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EVALUATION OF GENETIC VARIABILITY OF WILD MANGO VARIETIES OF DAKSHINA KANNADA AND UDUPI DISTRICTS OF KARNATAKA USING MORPHOLOGICAL AND MOLECULAR MARKERS

By

Ruchika
Reg. No. 166040309

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MANGALAGANGOTHRI – 574 199

Under the guidance of

Dr. RAMA BHAT P.

POST GRADUATE DEPT OF BIOTECHNOLOGY
ALVA'S COLLEGE,
MOODBIDRI -574227

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1. INTRODUCTION

Mango, a tropical fruit, is popularly known as the king of fruits (Mukherjee *et al*. 1957). It is also considered as the national fruit of India, Pakistan, and the Philippines and it is the national tree of Bangladesh. The scientific, botanical name for mango is *Mangifera indica* L.The term is probably derived from *mânkây* or *mangai*, the Tamil word for unripe fruit. It is likely that mânkây became *manga* in Malay and then, via Portuguese, it became mango in English. *Mangifera indica* was first described in botanical classification by Carl Linnaeus in 1753. These crops were valued for its delicious flavor, rich nutritional content, and diverse culinary applications (Majumber, 2011).

Mango (*M. indica*) is one of the most important and widely cultivated fruit crops in tropical and subtropical regions of the world with diverse morphological, physiological and biochemical traits (Roslin and Vinayagmurthy, 2014). It belongs to the family Anacardiaceae. Most of the mango varieties are dioecious and cross pollinated, and produce fertile hybrids. It is been cultivated in India for as long as 4000 years and reportedly originated in North-Eastern part of India. Vavilov (1926) has suggested Indo–Burmese (Myanmar) region for the centre of origin of mango (Mukherjee 1951, Singh *et al*. 2019). The cultivation of mango began in Malaysia and South-East Asia, probably spread by Buddhist monks. Phoenicians or Sumerians and Arabs took it from India to East Africa where it has been cultivated from the 10^{th} century AD onwards (Purseglove 1969). The widest distribution of mango around the world was carried out by the Portuguese by shipping the mangoes to western parts of the African continent and some parts of Brazil in South America (Jagarlamudi 2011).

According to Naik (1949) there are 82 varieties of mango grown in South India. Singh (1956) presented and illustrated 150 in their monograph on the mangoes of Uttar Pradesh. The wild mango varieties that exhibit significant morphological diversity in terms of fruit characteristics such as size, shape, color, texture, and taste. These wild varieties, often found growing in natural habitats and uncultivated areas, represent an important genetic resource for mango breeding programs aimed at developing improved varieties with desirable traits such as disease resistance, enhanced flavor, and extended shelf life. According to Zumajo-Cardona *et al*. (2019)**,** the leaf morphology may correspond to evolution through independent processes, such as branching, planation, webbing and overtopping. Based on these traits, this germplasm has low diversity. Wild mango varieties can have unique flavors and aromas that may differ from those of cultivated mangoes. Some wild varieties may have complex, tangy, or resinous flavors that are distinct from the sweet, aromatic taste of cultivated mangoes (Mursyidin, 2023).

The seeds of wild mango varieties can vary in size and shape, skin colors with some having larger or more irregularly shaped seeds (Dinesh *et al*. 2016; Joshi *et al.* 2016). Wild mango varieties may exhibit a wider range of skin colors, have rougher or more even skin textures and a broader spectrum of colors, ranging from green to yellow to red (Moretti-Almeida and Silva, 2016). Wild mango varieties have evolved to thrive in diverse ecological conditions. They may exhibit adaptations such as drought tolerance, disease resistance, and tolerance to various soil types, which may differ from those of cultivated mango varieties. Wild mango varieties contribute to the genetic diversity of the mango species, providing a valuable resource for breeding programs aimed at developing improved varieties with desirable traits such as disease resistance, drought tolerance, and enhanced fruit quality (Dwivedi *et al.* 2016).

Understanding the morphometric characteristics of fruits from different wild mango varieties is crucial for several reasons. It provides details about the genetic diversity present within the genus *Mangifera*, which can aid in the conservation and utilization of wild mango germplasm for future breeding efforts and it also helps to identify promising wild varieties with unique fruit traits that may be of interest for commercial cultivation or hybridization with cultivated mango varieties (Smith & Brown 2023).

To analyze the morphometric characteristics of fruits from various wild mango varieties, focusing on elucidating the range and extent of morphological diversity present within this diverse group of species. By employing measurement techniques and statistical analyses, majorly to uncover key differences in fruit morphology among different wild mango populations, thereby contributing to a better understanding of the genetic and phenotypic variability within the genus wild mangoes. Wild mango varieties share some characteristics with cultivated mangoes, they also exhibit unique traits that reflect their diverse origins, ecological adaptations, and genetic diversity. These differences make wild mango varieties important resources for both biodiversity conservation and the continued improvement of cultivated mango varieties through breeding programs (Rajan 2013).

The Birbal Sahni Institute of Palaeobotany, Lucknow has taken the origin of mango back to 60 million years' old fossil compressions of carbonized mango leaves in the period of Palaeocene, discovered in the region of Damalgiri, West Garo Hills of Meghalaya (Mehrotra *et al*. 1998). Indian peninsula, the most important centre of diversity for *Mangifera indica*, has a large array of cultivated varieties as well as wild types. There are 1000 mango varieties in India (Ganguli *et al*. 2019). Other species of *Mangifera indica* viz. *M. andamanica*, *M. khasiana*, *M. sylvatica* and *M. camptosperma* have been reported from India. *Mangifera sylvatica* occurs in the Eastern Himalayas (up to 1300 m altitude) and even in Sikkim, Darjeeling, Khasi Hills, Upper Assam and Surma Valley. The *Mangifera andamanica*, an endemic and rare species, is widespread in tropical, wet evergreen forests of Andaman Islands. *Mangifera indica* is therefore, distributed throughout the tropical and subtropical parts of India (Yadav 1999).

About 30 varieties are commercially grown in India including the Dakshina Kannada and Udupi districts of Karnataka. The wild mango varieties of Dakshina Kannada and Udupi districts are of great interest due to their unique and diverse characteristics including their flavor, aroma, and nutritional and medicinal values. These wild mango varieties are traditionally used by the local communities for their nutritional and medicinal properties. (Shah *et al.* 2010).

According to the Food and Agriculture Organization (FAO), the global mango production in 2020 was around 57.4 million tons and India was the leading producer accounting for around 40% of the total mango production (Makarabbi 2023).The importance of conserving mango genetic resources, especially the land races and wild species, cannot be over-emphasized (Warschefsky 2010) because the species is in danger of loss of its natural habitat.

The major mango growing states are Andhra Pradesh, Uttar Pradesh, Karnataka, Bihar, Gujarat and Telangana Andhra Pradesh ranks first in mango production with a share of 23.89% and the highest productivity in 2021-22 (3rd Advance Estimate). India is also a prominent exporter of Fresh Mangoes to the world. The country has exported 22693.76 MT of Fresh Mangoes to the world for the worth of Rs. 378.49 crores/48.53 USD Millions during the year 2022-23 (Agricultural and Processed Food Products Export Development Authority, Ministry of Commerce and Industry).

Bangladesh and the Indo-Myanmar area are where it is still grown and found in a wild form with tiny fruits. India produces more than half of the world's mangoes, making it the top producer in the world. With 192,000 hectares under cultivation and a 1.8 million ton annual

production, Karnataka is one of the states that lead the nation in mango production.The mango germplasm is being conserved through complementary in situ and ex situ strategies. However, a wealth of mango genetic resources is still poorly assembled and characterized (Rajan 2019). The list of states showing the cultivation of mango cultivars in India (Hand book of ICAR, 2002) given in table 1.

Molecular markers are widely used in *Mangifera* species for understanding genetic variation and to examine genetic relationship in Mango germplasm identification of duplicate accessions and genetic fidelity testing. Molecular markers were employed for characterization and genetic diversity analysis in Mango species including RFLPs (Gawell *et al*. 1992; Faure *et al*. 1994; Carreel *et al*. 2002), RAPDs (Williams *et al*. 1990; Schnell and Knight 1993), AFLPs (Loh *et al*. 2000; Ude *et al*.2002), microsatellite marker (SSR) (Ravishankar *et al*. 2015) and PCR-RAPD (Prithvish and Ckikkaswamy 2016). Among all these techniques microsatellite markers for mangoes are highly polymorphic with co-dominant mode of inheritance and are reproducible and easy to interpret.

Table 1: Lists of states showing the mango cultivars in India

State	Varieties grown
Andhra Pradesh	Allumpur Baneshan, Banganapalli, Bangalora, Cherukurasam, Himayuddin, Suvernarekha, Neelum, Totapuri
Bihar	Bathua, Bombai, Himsagar, Kishen Bhog, Sukul, Gulab Khas, Zardalu, Langra, Chausa, Dashehari, Fazli
Goa	Fernandin, Mankurad, Alphonso, Mulgoa
Gujarat	Alphonso, Kesar, Rajapuri, Vanraj, Jamadar, Totapuri, Neelum, Dashehari, Langra
Haryana	Dashehari, Langra, Sarauli, Chausa, Fazli
Himachal Pradesh	Chausa, Dashehari, Langra
Jharkhand	Jardalu, Amrapalli, Mallika, Bombai, Langra, Himsagar, Chausa, Gulabkhas
Karnataka	Alphonso, Bangalora, Mulgoa, Neelum, Pairi, Baganapalli, Totapuri
Kerala	Mundappa, Olour, Pairi
Madhya Pradesh	Alphonso, Bombay Green, Langra, Sunderja, Dashehari, Fazli, Neelum, Amrapalli, Mallika
Maharashtra	Alphonso, Mankurad, Mulgoa, Pairi, Rajapuri, Kesar, Gulabi, Vanraj
Orissa	Baneshan, Langra, Neelum, Suvarnarekha, Amrapalli, Mallika
Punjab	Dashehari, Langra, Chausa, Malda
Rajasthan	Bombay Green, Chausa, Dashehari, Langra
Tamil Nadu	Banganapalli, Bangalora, Neelum, Rumani, Mulgoa, Alphonso, Totapuri
Uttar Pradesh	Bombay Green, Dashehari, Langra, Safeda Lucknow, Chausa, Fazli
West Bengal	Bombai, Himsagar, Kishen Bhog, Langra, Fazli, Gulabkhas, Amrapalli, Mallika

Microsatellites (SSRs) are popular molecular markers because of their high abundance in the genome, co-dominant inheritance, the extent of allelic diversity, good reproducibility and the potential for high-throughout analysis and automatization. Simple Sequence Repeat (SSR) or microsatellite markers, offers a high-resolution approach to dissecting genetic variation within mango populations (Duval *et al*. 2005; Hadhary *et al*. 2015; Saleem *et al*. 2018; Sarika *et al*. 2019). The availability of a whole-genome sequence of a particular species enables the genome-wide identification and development of SSR

markers for that species(Cavagnaro *et al.* 2010; Song *et al*. 2015; Cheng *et al*. 2016). SSR analysis enables discrimination between individual species, subspecies and subgroups of mango accessions and is suitable for characterization of unknown accessions. This research work is an effort to characterize the morphological traits of the wild mango varieties and identify the variability among them; to assess the genetic diversity of the wild mango varieties using molecular markers and to compare the results obtained using morphological and molecular markers. It also aims at evaluating the usefulness in characterizing the wild mango varieties (Jena *et al*. 2021).

Objectives of the study

1. To analyse the morphometric characteristics of the leaf and fruits of different wild mango varieties of regions of Dakshina Kannada and Udupi.

2. To evaluate diversity among mango varieties using morphological characteristics.

3. To assess the genetic diversity and relationships among selected mango plants using SSR/microsatellite markers.

2. REVIEW OF LITERATURE

2.1 Different morphological characters were studied in Mango Research

Morphological characterstics an important trait that may be used to isolate one or more individuals from a group is known as a morphological marker. Plant breeding uses morphological indicators to choose an individual, such as plant height, leaf architecture, seed coat colour, blossom colour, fruit flavour, leaf scent etc.

The assessment of genetic diversity patterns in plant populations has made critical contributions in evolutionary biology, conservation genetics, and plant breeding and ecological genetics. Such assessments not only pertaining to the quantification and distribution of genetic diversity in plant population but also investigation of those processes that influence the distribution of genetic diversity. Plants in particular with their huge diversity in breeding system and contrasting life-histories, provide a rich source of information in relation to patterns and processes that characterize genetic diversity in populations. One of the technique commonly used, was methods to analyze physico-chemical changes during mango ripening: A multivariate approach was studied by Padda *et al*. (2011). The assessment of dry matter content, firmness, color, total soluble solids were made. The fruit firmness were determined by hand squeezing, TSS content in filtered juice from whole fruit tissue were less variable compared to juice squeezed by hand. Canonical discriminant analysis (CDA) was used to identify the best method to discriminate between maturity and ripening stages, acidity, TSS etc. There is strong evidence that morphological traits and molecular traits follows the similar patterns (Dinesh *et al*. 2013).

Kaur *et al.* (2003) conducted a study to compare the rheological, physicochemical, morphological and thermal properties of mango kernels from five different mango varieties namely, Chausa, Totapuri, Kuppi, Langra, and Dashehari. This is an important contribution to understand the properties of mango kernel starch, which is an underutilized source of starch in many parts of the world. It was found that the shape of the starch granules in mango kernels can vary from oval to elliptical, and the amylose content can vary from 9.1% to 16.3% in different mango varieties. Totapuri had the largest mean granular size and amylose content, while Chausa had the lowest mean granular size and amylose content. Dashehari starch paste showed lower turbidity values than other mango varieties.

Bhagwan *et al*. (2013) focused on investigating the impact of climate change on the productivity of Banganpalli mango in the Andhra Pradesh region of India during 2007 and 2010, and reported that the maximum and minimum temperatures during November to February had a significant influence on the productivity of Banganpalli mango. It was found that the temperature at night below 15°C during November-December greatly affected the productivity of Banganpalli mango. There was a sudden rise in temperature above 35°C during February, which resulted in delayed flowering, less number of perfect flowers, poor fruit set, and reduced productivity of Banganpalli mango was conducted at the Fruit Research Station, Sangareddy, where a progressive decrease in the number of fruits per tree was observed over the last four years of the study period and indicated that there was a significant decline in the number of fruits per tree from 2007 to 2010. The minimum number of fruits recorded during the study period was 273, which was similar to the number recorded in 2009.

Dinesh *et al*. (2013) conducted a study on the genetic diversity of indigenous mango varieties in Chittoor district, Andhra Pradesh, India. They selected 44 naati types from three communities belonging to 21 farmers, and evaluated them for various fruit characteristics, including fruit weight, length, and width, skin weight, stone weight, TSS, pulp percentage, and carotenoid content using Biodiversity International Descriptors (2016). They used SSR markers for molecular characterization and found that both morphological and molecular characterization followed a similar pattern, indicating that fruit characteristics were largely controlled by genetic factors. It is demonstrated that the diversity observed within a geographic region is derived from the varieties grown in that region.

Ribeiro *et al*. (2013) evaluated morphological characterization of mango (*Mangifera indica*) varieties based on Brazilian adapted descriptors on 103 mango varieties present in the germplasm collection. They evaluated various morphological characters of the mango varieties such as plant size, leaf size and shape, fruit size and shape, and seed morphology. They collected and evaluated 8 adult leaves, 8 flowers, and 16 fruits from the beginning four varieties and found that leaf symmetry and fruit waxiness did not show any significant variability among the varieties. The significant variations found on morphological traits such as fruit weight, length, width, and color of the epidermis, which ranged from green to red. The identification of varieties with high diversity in desirable traits can help breeders to develop new varieties with improved yield, resistance to diseases, and enhanced nutritional value.

Rosalin and Vinayagmurthy (2014) studied annual production and export of mango during 2014. Mango (*Mangifera indica* L.) grown on land covering 2,021 thousand acres.

India produced an estimated 12.5 million tons of mango, which accounted for 40.1% of the world's mango production. They focused the main mango-producing states in India, including Andhra Pradesh, Uttar Pradesh, Karnataka, Bihar, Gujarat and Maharashtra. The export market for mangoes from India, were 79.06 thousand tons in 2006-07, with a value of Rs. 141.9 crores. The mangoes are exported to over 50 different nations, with the majority (97%) of fresh mango exports going to the UAE, Saudi Arabia, Qatar, Bahrain, UK, Kuwait, Singapore, Malaysia and Bangladesh combined. The high percentage of mangoes lost during the harvest and post-harvest phases, which can be as high as 30-35%.

Kochummen's (2015) studied distribution and diversity of the *Mangifera* species. It was recorded 40 different species of *Mangifera* across the region, including India, Sri Lanka, Malaysia, Philippines and Papua New Guinea. The distribution of the species is widespread, and the highest diversity found in the Malayan Peninsula, Borneo and Sumatra. They reported that 30 different species have been recorded in Malaysia, demonstrating the rich diversity of this species in this region. It was noted that most of the *Mangifera* species are domesticated and cultivated in garden orchards or home gardens and promoting sustainable practices for the cultivation and conservation.

Ranjitha *et al*. (2016) evaluated the genetic diversity of 41 mango varieties in India using both morphological and molecular markers to identify unique and diverse genotypes for conservation and breeding. A total of 22 traits used for the morphological analysis including tree height, canopy shape, fruit weight, and fruit shape. It was revealed that significant variations are found in the morphological traits among the varieties. There were 10 SSR markers were used to analyze the genetic diversity of these 41 mango varieties and high level of genetic diversity among the varieties, with an average of 7.8 alleles per locus. There are four distinct clusters of mango varieties based on their genetic similarity.

Pillai *et al*., (2016) conducted morphometric analysis of 18 mango varieties in Sri Lanka using leaf, inflorescence and fruit characters to determine their food quality. Principal component analysis (PCA) and hierarchical cluster analysis (HCA) used to identify patterns and variations among the mango varieties. They reported that the mango varieties showed significant variations in both qualitative and quantitative traits, such as fruit shape, size, and color, which can have implications on consumer preference and commercial value. PCA and HCA were used to identify the most important traits that contributed to the variations observed among the mango varieties. The PCA revealed that the first three principal components accounted for 75.6% of the total variations, with fruit weight, fruit length, fruit diameter, and seed weight being the most important traits. HCA further grouped the mango

varieties into four clusters based on their morphological characteristics. The use of PCA and HCA allowed for a detailed analysis of the variations among the mango varieties, which can be useful for breeding programs and the commercial production of mangoes.

Naik and Kulkarni (2016) studied on morphological characterization of wild mango varieties in Karnataka with 28 morphological traits. The significant variability in fruit shape, size, color, skin texture, pulp color, seed shape, seed size, and seed coat color among the different varieties.

Ranjan *et al.* (2016) studied the genetic diversity of 42 important varieties of mango and their heritability and divergence in fruit characters by analyzing 14 quantitative fruit traits using genotypic and phenotypic coefficient of variation, heritability, genetic advance, and clustering using D2 with group constellation following Tocher's method. The results showed that weight of pulp, fruit, stone and peel, pulp stone ratio, length of fruit and stone had high genotypic coefficient of variation and heritability, indicating that these traits are important in breeding programs. The varieties were arranged into three distinct clusters based on their different morphological traits. The highest mean values for weight of fruit and peel, fruit length, width and thickness of fruit and stone, fruit length and width ratio (FLWR), and pulp: stone ratio were grouped in cluster III, while high pulp weight and per cent, TSS, weight, length, and width of stone were grouped in cluster II. The highest intra-cluster distance was observed in cluster I, while the highest inter-cluster distance was between cluster I and II and pulp weight contributed the most towards genetic divergence (34.03%), followed by peel weight (22.65%), TSS (10.22%), stone weight (7.90%), and width (5.46%). The lower genotypic coefficient of variation (GCV) than the phenotypic coefficient of variation (PCV) suggested that there is less environmental influence on the traits, but also indicates higher heritability which play a more significant role in efficient selection.

Yadav *et al.* (2016) studied mango cultivation across the world, with a particular focus on India, accounts for the largest share of mango production globally, with 46.75% of the world's land devoted to mango cultivation. They supported by evolutionary and taxonomic studies, which demonstrate the prevalence of mango cultivation across tropical Asia, including the Northeast region, Myanmar and Bangladesh. The global commercial importance of mangoes, with more than 90 countries engaged in mango production. Mangoes are known to contain 70 species, all of which are confined to tropical Asia. India is the largest producer of mangoes, with a significant portion of its population involved in the production, distribution, and marketing of this fruit.

Tekalign *et al.* (2017) evaluated the genetic diversity of mango 15 varieties in Ethiopia using both morphological and molecular markers to assess the extent of genetic variation among mango varieties in Ethiopia using 13 traits, including tree height, canopy shape, fruit weight, and fruit shape. The analysis revealed significant variations in the morphological traits among the varieties. The SSR markers to analyze the genetic diversity of the same 15 mango varieties. The molecular analysis revealed a moderate level of genetic diversity among the varieties, with an average of 5.38 alleles per locus. They identified three distinct clusters of mango varieties based on their genetic similarity. The combination of morphological and molecular markers are used to obtain a assessment of the genetic diversity of mango varieties in Ethiopia.

Alam *et al*. (2017) evaluated the genetic diversity of 26 mango varieties in Bangladesh using both morphological and molecular markers to assess the extent of genetic variation among mango varieties in Bangladesh using 10 traits were used including tree height, canopy shape, fruit weight, and fruit shape, to evaluate 26 mango varieties. The analysis revealed significant variations in the morphological traits among the varieties. For the molecular analysis, 16 SSR markers were used with an average of 6.13 alleles per locus and identified four distinct clusters of mango varieties based on their genetic similarity.

Singh *et al*. (2017) studied on the morphology and physical attributes of 43 mango varieties, majorly on the variations in fruit size, weight, pulp stone ratio, fiber content, and other surface morphological features conducted by the Horticulture Research Centre, G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, over two years (2012-2014) using a randomized block design method. The significant variations among the different mango varieties in terms of fruit shape, weight, size, pulp stone ratio, fiber content, and density of lenticels. The Haathijhool variety emerged as the most promising in terms of fruit weight, size, and pulp stone ratio, with a high fiber content of 5.35%, making it the most fibrous among all the varieties tested. The high pulp stone ratio of Haathijhool (10.50) in both years of the study also makes it suitable for fruit processing, as concluded by Mitra *et al*. (2001). However, the lowest pulp-to-stone ratio observed in the Kesar (Basti) variety in the first year of research and in the K.O.-07 variety in the second year, focused the variability of fruit characteristics among different mango varieties and pooled analysis of data also showed significant variations in the waste index of mango varieties, with Haathijhool and Mallika having the highest waste index values of 5.27 and 4.29, respectively.

Dinesh *et al.* (2018) evaluated among mango grown in Kuttiattoor, Thaliparamba Tehsil, Kannur District of Kerala. The unique characteristics traits in genetic, morphological,

and biochemical characteristics compared to other mango varieties. This uniqueness was further supported by the DNA barcoding analysis conducted by Li *et al.* (2015), which was showed that the DNA barcodes of 10 varieties were different from one another and suggests that the Kuttiattoor mangoes are a distinct variety and qualify for registration with the Protection of Plant varieties and Farmers' Rights Authority. The significance of kuttiattoor mangoes mainly contribute to the preservation and protection of agricultural biodiversity.

Thakur *et al.* (2019) conducted investigation of the fruit quality of wild mango fruit in various locations in Himachal Pradesh. The seedling mango has gained economic importance due to its use in pickles and other food preparations. They used cluster analysis (SPSS) to evaluate the physical, chemical, sensory, physico-chemical, and combined physical-chemical and sensory characteristics of the seedling mango fruits collected from 24 different sites in six districts of Himachal Pradesh. They found notable variations in the fruit quality attributes from location to location. Based on the quality characteristics including higher acid-edible content indicator (2.36) and sensory quality, fruits of 10 locations were found suitable for pickle preparation. These locations were Kangra (Sulah and Bhawarna), Una (Una Sadar and Amb), Hamirpur (Bhoranj), Bilaspur (Ghumarwin), Mandi (Gopalpur and Pandoh), and Sirmour (Nahan and Ponta Sahib).

Vijayalaxmi and Periasami (2020) studied on the production and marketing of mangoes in the Krishnagiri district in relation to variety of nutrients, including vitamin B, carotene, carbs, vitamin "C," fiber, and energy. Its sophisticated flavor and wide range of attributes make it the monarch of all fruits in the globe. Bangalora, Badami, Banganapalli, Kesar, Alphonsa, and Dashehari are the main export kinds. Mango production is substantially aided by the fact that they are mostly used to create refreshing and sweet beverages. Water is not needed during monsoon seasons since irrigation is the primary source of requirement for mango agriculture.

Nascimento *et al*. (2020) investigated the "Morphological and molecular characterization of 24 different mango varieties grown in Brazil, using both morphological and molecular techniques using 24 traits. The molecular analysis used 11 microsatellite markers, which ensured high levels of accuracy and reproducibility in the identification of the different mango varieties. Mango varieties were grouped into four major clusters based on their morphological traits, which corresponded well with their geographical origin. The molecular analysis also confirmed the morphological grouping, with high levels of genetic diversity observed within and between the different groups.

Gunathilake *et al*. (2020) evaluated genetic diversity of 15 mango varieties in Sri Lanka using both morphological and molecular markers (RAPD markers) to assess the genetic diversity. The results exhibited a high level of morphological diversity among the 15 mango varieties, with variations observed in fruit shape, size, color, and texture. They found significant differences in the RAPD marker profiles of the mango varieties, indicating a high level of genetic diversity. They used cluster analysis to evaluate the genetic relationships among the mango varieties and found that the mango varieties were divided into two main clusters, which were not correlated with their geographic origin.

Akhter *et al*. (2021) reviewed the morphological, nutritional, and medicinal attributes of wild mango (*Mangifera Sylvatica* Roxb.), The morphological characteristics of wild mango in comparison to cultivated varieties, emphasizing its potential as a genetic resource for varieties development. Nutritional analysis reveals the richness of essential nutrients in wild mango, suggesting its suitability as a valuable food source. The medicinal properties of wild mango, including its antioxidant and antimicrobial activities, indicating its potential applications in healthcare and pharmaceutical industries. The genetic diversity, opportunities emerge for the development of improved varieties with enhanced traits suited for agricultural and medicinal purposes.

Sridhar *et al.* (2022) worked in All India Coordinated Research Project on Fruits in Gayeshpur, West Bengal, from 2016 to 2018, examined the plant, leaf, inflorescence, fruit, stone, and seed characteristics of 16 mango varieties. Morphological characterization was performed based on IPGRI descriptors for mango, encompassing qualitative and quantitative traits. Significant variations were observed across various parameters such as crown shape, young leaf color, leaf blade dimensions, flowering regularity, inflorescence characteristics (including color, length, and width), pulp color, and texture. The Kamala Bhog exhibiting the maximum inflorescence length (45.50 cm) and width (31.74 cm). Gopi Bhog displayed the highest stone weight (46.40 g), while Gopal Bhog had the highest seed weight (22.85 g). There is variations in genetic makeup among the varieties.

Shivran *et al.* (2023) studied on "Morphological Characterization of Mango (*Mangifera indica* L.) Germplasm under Tarai Region". The morphological assessment of mango germplasm present in this region using a combination of qualitative and quantitative morphological parameters to characterize and classify different mango varieties. The germplasm PMSS-9 exhibited longest flowering duration. The secondary/ off-season flowering was absent and terminal position and semi erect inflorescence axis growth habit were observed in all the mango germplasm. The variations in fruit size, shape, color, and

other key characteristics among the mango germplasm, contributing to our understanding of local biodiversity.

2.1 Mango Genetic Diversity

Indian peninsula, the most important centre of diversity for *Mangifera indica,* has a large array of cultivated varieties as well as wild types. There are 1000 mango varieties in India (Ganguli *et al*. 2019). Other species of *Mangifera indica* viz. *M. andamanica, M. khasiana, M. sylvatica* and *M. camptosperma* have been reported from India. *Mangifera sylvatica* occurs in the Eastern Himalayas (up to 1300 m altitude) and even in Sikkim, Darjeeling, Khasi Hills, Upper Assam and Surma Valley. The *Mangifera andamanica*, an endemic and rare species, is widespread in tropical, wet evergreen forests of Andaman Islands. *Mangifera indica* is therefore, distributed throughout the tropical and subtropical parts of India (Yadav 1999).

About 30 varieties are commercially grown in India including the Dakshina Kannada and Udupi districts of Karnataka. The wild mango varieties of Dakshina Kannada and Udupi districts are of great interest due to their unique and diverse characteristics including their flavor, aroma, and nutritional and medicinal values. These wild mango varieties are traditionally used by the local communities for their nutritional and medicinal properties. (*Shah et al.* 2010)

2.2 Different Marker Technology Applied in Mango Research

2.2.1 Biochemical Markers

Biochemical markers are the molecules or the products of genes such as proteins/enzymes which are polymorphic in their structure as well as their amino acid composition. The isozymes are encoded by the homologous gene located in the different loci in the genome, while allozymes are allelic variants of the same gene. Allozymes and isozymes move at different speeds through a gel because they differ from each other in size, structure, and charge. So, allelic variation can be detected among the individual in a population by using this type of marker. Mango have a partial allopolyploid genome based on cytogenetics (Mukherjee, 1950). However, genetic markers for mango have been reported to be inherited in a disomic fashion by several authors (Duval *et al.*, 2005; Schnell *et al*., 2005, 2006; Viruel *et al*., 2005) suggesting that mango is treated as diploid. Mango has a total

of 40 chromosomes, which suggests a haploid number of chromosomes as 20 and similarly 20 linkage groups. The biochemical markers are sensitive to the developmental, environmental issues and have a reproducibility problem.

Rao *et al.* (2017) evaluated on biochemical composition of several mango varieties grown in the state of Karnataka by several analytical techniques such as HPLC, GC-MS, and spectrophotometry. They identified the variations in the chemical composition of these mango varieties, which could help in identifying the suitable variety for processing and export purposes It was found that the mango varieties differed significantly in their biochemical composition, with variations in the levels of sugars, acids, vitamins, minerals, and phytochemicals. They have identified several new phytochemicals that were not previously reported in mangoes.

Aswini and Bhaskar (2023) studied the morphological and biochemical characterization of mango (*Mangifera indica* L.) genotypes under high-density planting systems is pivotal for modern mango cultivation. High-density planting, a strategy to maximize yield per unit area, necessitates careful selection of mango genotypes tailored for this system. Morphological assessment encompasses growth habit, tree size, leaf and fruit traits and flowering patterns. Genotypes exhibiting compact growth, manageable canopy sizes, consistent flowering, and desirable fruit characteristics are deemed suitable for high-density orchards. Biochemical analysis focused on sugar content (Brix), acidity (pH), and nutrient composition, including vitamins, minerals and antioxidants. Secondary metabolites like phenolic compounds and flavonoids are also considered, as they contribute to fruit flavor and potential health benefits. Understanding enzyme activities during ripening and post-harvest stages aids in optimizing storage and shelf life.

Saroj *et al.* (2023) characterized the bioactive and fruit quality compounds of promising mango genotypes grown in the Himalayan plain region for mango cultivation. The need for sustainable fruit production in challenging terrains like the Himalayas and its foothills were noted. Mangoes are an integral part of the region's horticultural landscape, and understanding the bioactive compounds in these fruits is vital. The presence of varied concentration of secondary metabolites, including phenolics, flavonoids, and antioxidants, in different mango genotypes not only influence fruit flavor and aroma but also have significant health benefits, making them essential for consumer preferences. The evaluation of fruit quality parameters such as sugar content, acidity, vitamin levels and mineral compositions are into the nutritional value and overall desirability of these mango genotypes.

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2.2.2 DNA Markers

Diversity among organisms is a result of variations in DNA sequences and environmental effects. Genetic variation in substantial and each individual of a species, with the exception of monozygotic twins, possesses a unique DNA sequences. Information on genetic diversity is essential in opting both conservation and utilization strategies and number of markers are available to detect polymorphism in nuclear DNA. Molecular characterization can play a role in uncovering the history, and the estimating the diversity, distinctiveness and population structure. It can also serve as an aid in the genetic management of small populations, to avoid excessive inbreeding.

Dillon *et al.* (2013) studied the genetic diversity of the 50 Australian mango gene bank using molecular markers. It was revealed that there were 22 unique genotypes among the trees, while the remaining accessions were genetically distinct from each other. The identification of the unique genotypes will help the breeders to select parents with the potential to contribute desired genes to the progeny such as disease resistance, fruit quality, yield, and shelf life. The genetic diversity identified would help to ensure the long-term sustainability of the mango industry in Australia. The importance of maintaining a gene bank for mangoes and gene banks are essential for the conservation of genetic diversity and the preservation of genetic resources.

2.2.2.1 Molecular Evidence of Evolution Using DNA Markers

Several molecular methods were adopted to analyze diversity and evolution in the early 1980s, As soon as DNA markers along with PCR based techniques were available, several markers like RFLP, AFLP, RAPD, ISSR, and SSR microsatellite markers are being used to analyze diversity across different countries and research groups with their germplasm collections.

2.2.2.2 Restriction Fragment Length Polymorphism (RFLP)

RFLP markers are often used in combination with other types of markers, such as RAPD, SSR, etc., in mango breeding although RFLP markers are not suitable for large scale utilization in the breeding program because of the complicated assay technique, expensive nature, and require sophisticated equipment's.

Naem *et al.*(2010) conducted the genetic diversity of 20 mango cultivars in Pakistan using five Amplified Fragment Length Polymorphism (AFLP) primer combinations of markers to identify the genetic relationship among different mango cultivars and to determine the potential of AFLP markers in assessing the genetic diversity of mango germplasm. It was showed high levels of genetic diversity among the cultivars, with a total of 534 bands being generated, of which 527 were polymorphic. The average number of bands per primer combination was 106.8, indicating a high level of genetic variation.

Rahman *et al.* (2022) studied on the molecular characterization of local mango germplasm in Chapainawabganj, Bangladesh in which they employed various molecular techniques, including molecular markers (such as SSRs, AFLPs, and RAPDs) and DNA sequencing, to assess the genetic diversity among local mango germplasms. This provided a rich genetic resource of mango varieties with distinct genetic profiles which is crucial for breeding programs aimed at improving disease resistance, fruit quality, and adaptability to changing environmental conditions. Several studies have focused on identifying and characterizing specific traits, such as fruit size, color, taste, and disease resistance, through molecular markers. The molecular characterization has been instrumental in conserving endangered or rare local mango varieties, ensuring their preservation for future generations and safeguarding biodiversity.

2.2.2.3 Variable Number of Tandem Repeats (VNTR)

Jena *et al* (2021) evaluated the genetic diversity of 70 Indian mango genotypes based on geographic origin and fruit status using multiple DNA markers. The results showed that the East Indian population had the highest genetic variation, followed by South India, West India, and North India. The local genotypes of Odisha, which belongs to the East Indian population, exhibited the highest diversity compared to the hybrid or selection groups, indicating their potential for exploiting unique and favorable alleles and used various DNA markers such as RAPD, ISSR, DAMD, SCoT, CBDP, and SSR to examine the level of genetic polymorphism and diversity within and among the mango genotypes. The maximum genetic variability was detected in geographical/fruit status populations with SSRs, followed

by CBDPs and SCoTs. These markers are useful for assessing the genetic diversity of mango germplasm and could facilitate conservation and further exploitation of mango genetic resources through breeding. The results of AMOVA analysis indicated a greater amount of intra-population genetic variation compared to heterogeneity at the inter-population level.

Lowe *et al* (2000) studied the conservation genetics of bush mango from central or West Africa using RAPD analysis with 130 accessions and 74 RAPD markers to explore the genetic variation within and between populations of the species. The significant genetic differentiation was found between the populations of bush mango. Among species analysis of molecular variance (AMOVA) was used to evaluate the genetic variation, and the significant variance component was found to be 25.8%, with a p-value of less than 0.001. This indicates that there is substantial genetic variation within the species, which is important for conservation efforts. There is a possibility of hybridization between the two species of bush mango, and found that the significant variance component was 9.8%, with a p-value of less than 0.001. This suggests that hybridization between the species is occurring, which could lead to the creation of new genetic variation

2.2.2.4 Random Amplified Polymorphic DNA (RAPD)

Schnell *et al*. (1995) investigated the identification of cultivars and genetic relatedness in *Mangifera indica* using RAPD techniques. They examined 25 accessions of mango using 80 primers, of which 33 did not get analyzed, 19 were monomorphic, and 28 gave desired results of variations. The results showed that the RAPD technique was able to provide reliable information about genetic diversity and relationships among the accessions studied. The use of RAPD technique has several advantages, including its simplicity, cost-effectiveness, and the ability to generate DNA fingerprints that can be used for comparative analyzes of genetic relationships among individuals.

Ravishankar *et al*. (2000, 2004) and Karihaloo *et al.* (2003) used Random Amplified Polymorphic DNA (RAPD) markers to investigate the genetic relatedness among different mango cultivars in India. RAPD markers are PCR-based markers that allow for the amplification of random DNA fragments, providing a unique pattern of DNA bands that can be used to assess genetic diversity. Ravishankar *et al*. (2000), studied the genetic relatedness among few Indian mango cultivars using RAPD markers. Their results revealed that genetic similarities among the samples decreased with an increased geographical distance. This suggests that the genetic diversity of mango cultivars is influenced by their geographical origin. In Karihaloo *et al*. (2003), they investigated the diversity of six mango populations of

Colombo cultivars using RAPD markers. Ravishankar *et al*. (2004), the genetic relatedness among polyembryonic and monoembryonic mango cultivars traditionally grown on the West coast of Southern India using RAPD markers was studied.

Arogundade *et al*. (2005) analysed the genetic diversity among mango cultivars using 16 SSR markers. The use of molecular markers, particularly PCR-based SSR markers with high level of polymorphism with 35-100% genetic similarity among the 16 mango cultivars. The one-way ANOVA and correlation using PCA, to analyze the morphological and genetic data. The results of the PCA analysis showed a strong correlation between fruit length, width, thickness, pulp percentage, and stone percentage. The dendrogram analysis revealed a similarity coefficient of 48-93%, indicating a high level of variability and outbreeding among the mango cultivars. Dandey (1998) and Krishna and Singh (2007) reported the outbreeding ability of mango and the resulting genetic diversity within the crop. Shamili *et al*. (2005) also found a high level of genetic similarity among 41 mango cultivars using 16 SSR markers.

Srivastav *et al*. (2014) conducted a study on the isolation of genomic DNA from mango (*Mangifera indica* L) for next-generation sequencing using random amplified polymorphic DNA (RAPD) markers. They have been successfully isolated high-quality DNA from young leaves of mango and optimized the RAPD protocol for generating informative genetic fingerprints. The DNA extraction, quantification and quality assessment were carried. The results showed that the optimized RAPD protocol was effective in generating reproducible and informative fingerprints, with 90% of the primers producing amplification products. They also used the generated RAPD fingerprints to analyze the genetic diversity and relatedness of different mango cultivars.

Karibasappa *et al*. (2014) evaluated the genetic diversity of 20 wild mango varieties from different regions of Karnataka using RAPD markers. The high levels of genetic diversity within the wild mango populations, with a total of 188 bands detected across all the samples, of which 178 were polymorphic. The results showed that the wild mango populations clustered based on their geographic origin, suggesting that the genetic diversity of these populations is influenced by their geographic distribution.

Trivedi *et al*. (2015) conducted a study on the effect of biofield energy treatment on the morphological characteristics, quality, yield, and DNA fingerprinting of Alphonso mango (*Mangifera indica* L.). The research was carried out on a 16-acre plot that was divided into two sections one was treated with biofield energy while the other was kept as a control. The results showed that the biofield energy treatment had a significant effect on the morphology of the mango trees. The leaves of the treated trees were found to be straight and healthy,

whereas the control group showed infected, curly, and distorted leaves. Additionally, the fruit weight was found to be higher in the biofield energy-treated trees compared to the control group. The quality assessment of the treated mangoes also revealed significant differences. The pulp and vitamin C content of the treated Alphonso mangoes were found to be higher than those of the control group. This suggests that biofield energy treatment can enhance the quality of the fruit produced. The DNA fingerprinting data using RAPD (Random Amplified Polymorphic DNA) analysis revealed that the treated samples did not show any true polymorphism when compared to the control group. This suggested that the biofield energy treatment did not cause any genetic modifications in the mango trees.

Malik *et al*. (2015) evaluated the genetic diversity of 42 mango varieties from different regions of India using molecular markers showed a high level of genetic diversity among the studied mango varieties, indicating a vast pool of genetic resources that can be utilized for developing new and improved varieties. It also showed that the genetic distance among the varieties was generally low, indicating a close genetic relationship among the samples. They noted that some of the varieties had distinct genetic profiles, suggesting that they were either of different origin or had undergone significant genetic modification. The cluster analysis based on the genetic data and found that the varieties could be grouped into four major clusters. The grouping was largely based on the geographical origin of the varieties, with most of the varieties from the same region clustering together.

Kumar *et al*. (2015) evaluated the genetic diversity of 15 mango germplasm in the Western Himalayan region of India using 20 RAPD primers. It showed a high level of genetic diversity among the mango cultivars, with the RAPD markers being effective in differentiating between closely related cultivars. They identified a total of 237 RAPD markers, of which 221 were polymorphic, indicating a high degree of genetic diversity in the mango cultivars. This indicated the presence of unique genetic markers in some of the mango cultivars, with potential for developing new cultivars with desired traits.

Ahmed *et al.* (2015) conducted a study on the diversity of 30 mango cultivars in Shendi area of Sudan, of which sixteen cultivars were grafted genotypes of Indian or Egyptian origin. They assessed the intra-specific diversity of mango varieties in the region. The study showed a high level of intra-specific diversity among the mango cultivars. They found significant differences ($p < 0.05$) in the size, weight, and circumferences of the fruits among the three genotypes. However, no significant differences were observed in the fruit shape, apex shape, and other characteristics but there is a significant level of genetic variability in the mango cultivars.

Choudhary (2016) conducted a study to assess the genetic diversity of mango (*Mangifera indica* L.) germplasm using 20 RAPD primers to determine the genetic diversity and relationship among different mango cultivars and to identify unique genetic traits for their conservation and breeding programs. They found a high level of genetic diversity among the germplasm accessions, with 178 alleles detected across the primers. The average number of alleles per primer was 8.9, indicating a high level of genetic variability with an average polymorphism information content (PIC) value of 0.57. The genetic distance analysis showed that the germplasm accessions were grouped into three major clusters based on their geographic origin. It was revealed that a significant association between the genetic diversity of mango germplasm and their fruit quality traits, such as fruit weight, pulp thickness, and TSS content.

Kumar *et al*. (2016) studied the genetic diversity of 20 wild mango varieties from the Western Ghats region of Karnataka, India using RAPD markers. The RAPD markers are based on PCR amplification of random segments of genomic DNA, which results in the amplification of fragments of varying lengths. Banding patterns and it showed a high level of genetic diversity among the wild mango varieties. The detection of 287 bands across all the samples, with an average of 14.35 bands per primer. The percentage of polymorphic bands was 96.86%, indicating a high level of genetic variation. The genetic similarity coefficient among the samples ranged from 0.19 to 0.84, with an average of 0.48, indicating moderate genetic diversity among the samples. They performed cluster analysis and dendrogram showed three distinct clusters, with some samples showing a high degree of genetic similarity within the clusters.

Saharan *et al*. (2016) evaluated the genetic diversity of 13 wild mango varieties from Rajasthan, India using 10 RAPD markers. The PCR amplification of genomic DNA using RAPD primers produced a total of 194 bands, out of which 182 were polymorphic, indicating a high level of genetic diversity among the wild mango varieties from Rajasthan. The cluster analysis, genetic diversity analysis, and principal coordinate analysis (PCoA). Cluster analysis based on the genetic similarity coefficients revealed three distinct clusters, with some samples showing a high degree of genetic similarity within the clusters. The genetic diversity analysis showed that the observed heterozygosity ranged from 0.06 to 0.22, and the expected heterozygosity ranged from 0.14 to 0.26, indicating moderate to high levels of genetic diversity among the wild mango varieties from Rajasthan. The PCoA results showed that the first two principal coordinates accounted for 47.5% of the total variation, indicating a high level of genetic diversity among the samples.

Prithvish and Chikkaswamy (2016) investigated the genetic relationships among 19 different mango varieties using a PCR-based molecular marker method RAPD. There is an average, 5-6 discrete bands/varieties were yielded with 10 nucleotide primers. The size of the amplified products ranged from 100-3500 base pairs in length, and an average of 5-10 bands per primer were obtained. Of the 0.0621 amplified fragments, 50 were polymorphic (80%) with at least one pairwise comparison between 19 cultivated varieties . The RAPD technique used in the study is a simple and efficient method for detecting DNA polymorphisms and has been widely used in genetic studies. The technique is based on the use of a short, random sequence primer to amplify multiple regions of DNA simultaneously.

Mahato *et al.* (2017) evaluated the genetic diversity of 31 wild mango varieties from six different regions of India using 12 RAPD markers. They used PCR amplification to amplify the DNA of the mango samples and analyzed the resulting banding patterns. It showed a high level of genetic diversity among the wild mango varieties. It detected with a total of 191 bands across all the samples, with an average of 15.91 bands per primer. The percentage of polymorphic bands was 92.1%, indicating a high level of genetic variation. The genetic similarity coefficient among the samples ranged from 0.28 to 0.86, with an average of 0.58, indicating moderate to high genetic diversity among the samples. The dendrogram showed four distinct clusters, with some samples showing a high degree of genetic similarity within the clusters.

Singh (2019) evaluated the genetic diversity of mango germplasm using both morphological and molecular markers. The morphological and molecular markers to evaluate the genetic diversity of mango accessions and the analysis of morphological data revealed significant variation in fruit weight and pulp texture, while the analysis of molecular data using SSR markers revealed a high level of genetic diversity. The dendrogram provided a visual representation of the relationships between different mango accessions.

Bhattacharya *et al*. (2016) investigated the genetic diversity of 34 mango cultivars in West Bengal, India, using 20 RAPD markers revealed with high genetic diversity among the cultivars, with a total of 190 alleles being identified, of which 181 were polymorphic. The average number of alleles per locus was 9.5, indicating a high level of genetic variation. The results of could be used in the development of improved mango cultivars with desirable traits such as disease resistance, higher yield, and improved fruit quality.

Kyaing *et al.* (2019) evaluated the genetic diversity of mango varieties in Myanmar using 30 decamer operon primers to carry out the genetic analysis and construct an arithmetic mean dendrogram based on a cophenetic ratio of 0.9732. It is majorily to identify unique

polymorphic bands among varieties and develop SCAR primers for cultivar improvement in breeding programs. The dendrogram constructed from the data was also informative, providing a visual representation of the relationships between the different varieties studied. These primers can be used to identify specific genetic markers associated with desirable traits in mango cultivars, which could be useful in breeding programs to improve the quality and productivity of mango crops.

Mahmoud *et al*. (2019) conducted a study to analyze the genetic diversity of 27 mango cultivars in Egypt using 10 ISSR and RAPD markers. Their study revealed a high level of genetic diversity among the cultivars, with 66 alleles detected across the 18 markers. The average number of alleles per locus was 3.67, indicating a moderate level of genetic variability. revealed a high level of polymorphism, with an average polymorphism information content (PIC) value of 0.39. The mango cultivars in Egypt were genetically diverse, indicating the importance of preserving them for their unique genetic traits. The genetic distance analysis showed that the cultivars were grouped into four major clusters based on their geographic origin. There is a significant correlation between geographic distance and genetic distance, indicating the presence of genetic differentiation among mango populations in Egypt.

Maurya *et al.* (2022) studied the genetic diversity in mango (*Mangifera indica* L.) based on RAPD markers. RAPD markers have identified unique genetic fingerprints for various cultivars, demonstrating the rich genetic reservoir within the *Mangifera indica* species. This diversity is pivotal for breeding programs aimed at developing mango varieties with improved traits such as disease resistance, fruit quality, and adaptability to varying environmental conditions. The RAPD-based assessments have revealed the presence of clonal variants and helped in the detection of duplicate accessions within germplasm collections.

2.2.2.5 Inter simple Sequence Repeats (ISSR)

Karibasappa *et al*. (1999) studied the characterization of mango germplasm in North Karnataka using cluster analysis. The method used was a numerical taxonomic approach of unweighted pair group method using arithmetic average to cluster 67 genotypes based on 37 quantitative and 6 qualitative characters. There were 11 clusters, the use of hierarchical clustering techniques in this allows for the identification of patterns in the data, which can be used to group similar genotypes together. Canonical roots analysis used after standardization of data matrix to further analyze the results of the cluster analysis. The use of canonical roots analysis is a useful statistical method that can help identify the underlying relationships

between variables and highlight important patterns in the data. The first three vectors accounted for 78.1% of the variation in the data, which indicates that these vectors are important in explaining the genetic diversity of mango germplasm in North Karnataka.

Eiadthong *et al.* (2000) evaluated on evaluating the genetic relationships among 14 *Mangifera* species in Thailand using 217 AFLP markers. The results obtained through UPGMA and NJ trees showed that AFLP markers can be useful in assessing the diversity of germplasm collection of *Mangifera* species. The common mango was related to other species such as banana mango (*M. sylvatica* Roxb.), *M. laurina*, and *M. oblongifolia* Hook.f., *macrocarpa*, *M. foetida*, and *M. odorata*, which belong to subgenus Limus, were related to *M. indica* in both UPGMA and NJ trees. This finding is significant because these species are classified differently from the subgenus *Mangifera* to which *M. indica* belongs. The *M. gedebe* Miq. and *M. griffithii* Hook.f. were placed in distant positions among the *Mangifera* species tested and these two species are distantly related to *M. indica*.

Xu *et al.* (2000) investigated the rates of expansion and contraction mutations in simple sequence repeat (SSR) loci and how they vary with the length of the repeat. It was found that the rate of expansion mutations remained constant for all loci, whereas the rate of contraction mutations increased exponentially with SSR repeat length. The validation of polymorphic information content (PIC) value in allelic variation studied. A higher PIC value indicates a greater degree of allelic variation, which can be useful for distinguishing between different genotypes. Several subsequent studies, including those by Shareefa (2008) and Nayak (2010), have shown that SSR markers with a greater polymorphism percentage and consistent amplification pattern can be highly suitable for mango genotypes. It was reported that a set of 145 SSR markers with repeat lengths between 51-70 had a success rate of 88.96%, The dynamics of SSR mutation, which can be useful for developing SSR markers for genetic analysis. The high success rate of the set of 145 SSR markers highlights the potential of SSR markers for genetic analysis in mango research.

West *et al.* (2002) investigated the performance of direct fluorescent primers compared to M13 tailed primers in detecting microsatellites in *Pinus taeda*. They used an automated system that allowed them to load multiple dyes at once, each with a different emission wavelength, and multiple PCR product sizes. This approach enabled them to obtain high-resolution results that were reliable and reproducible. The direct fluorescent primers were superior to M13 tailed primers. The direct fluorescent primers were more efficient in detecting microsatellites, and they showed better resolution, with fewer mispriming or suboptimal PCR amplification events leading to the absence of amplification. They compared

the performance of the primers based on the IR2 gene, using the IRTM 4200 DNA analyzer from LI-COR, Lincoln, NE, USA. The PCR products showed a specific product size and inheritance pattern, and the standard PAGE gel was used for analysis. The direct fluorescent primers are preferable to M13 tailed primers in detecting microsatellites in *Pinus taeda*, which could have implications for other organisms with similarly repetitive DNA sequences.

Gajera *et al*. (2011) studied on the genetic variability and relationships among 20 *Mangifera indica* genotypes representing 15 endangered and 5 cultivars, obtained from Indian Gir forest region and were analyzed using 10 RAPD and 21 inter simple sequence repeat (ISSR) markers. RAPD markers were more efficient than the ISSR assay with regards to polymorphism detection. The average numbers of polymorphic loci per primer, average polymorphic information content (PIC) and primer index (PI) values were more for RAPD than for ISSR. The total number of genotype specific marker loci, Nei'ls genetic diversity (h), Shannon's information index (I), total heterozygosity (Ht), average heterozygosity (Hs) and mean coefficient of gene differentiation (Gst) were more for ISSR as compared to RAPD markers. The regression test between the two Nei's genetic diversity indexes showed low regression between RAPD and ISSR based similarities but maximum for RAPD and RAPD + ISSR based similarities.

Vasugi *et al*. (2012) analyzed the genetic diversity and chemical characteristics of indigenous mango accessions, specifically the 'Appemidi' type, in the Western Ghats region. They used 14 Simple Sequence Repeats (SSR) markers developed at the Indian Institute of Horticultural Research in Bangalore to assess genetic diversity among 43 mango genotypes. The clear differentiation between the mango genotypes, with two main clusters identified based on their fruit characteristics. Cluser I included highly acidic types, while cluster II comprised less acidic but higher TSS groups. The clustering was based on the primary qualitative characteristics of TSS, titrable acidity, pH and sugar. They analyzed the volatile chemicals responsible for each variety's distinctive aromas using gas chromatography-mass spectrometry (GCMS). They identified 285 volatile chemicals, including monoterpenes, sesquiterpenes, esters, aldehydes, ketones, alcohols, acids, aliphatic hydrocarbons, and aromatics, in various mango cultivars.

Singh *et al.* (2012) studied into the genetic diversity of mango hybrids using SSR markers. The results show that SSR markers are highly polymorphic, with an average of 3.47 alleles per primer. The alleles varied in size from 100 to 480 bp, indicating a high level of variation among the mango hybrids. The Jaccard's Similarity Coefficient values revealed a wide range of genetic diversity among the mango hybrids, ranging from 0.38 to 0.97. This

indicated that SSR markers are suitable for assessing genetic diversity and relationships among mango hybrids. The UPGMA cluster analysis-based dendrogram divided the mango hybrids into three main groupings, with the five most diversified hybrids forming Cluster 'A'. The hybrid H-9-6 had two distinct fingerprints, indicating the presence of two genetically different plants. The Principal Coordinate Analysis showed that mango hybrids tended to cluster, indicating a higher affinity for the female parent, Amrapali. The use of marker-assisted selection (MAS) can reduce the number of backcross generations required for gene introgression, leading to a more efficient and cost-effective breeding process.

Uddin *et al*. (2014) compared the standard CTAB method with a modified protocol that involved the use of 0.4 M glucose. The modified protocol proved to be superior in several ways, including the removal of major contaminants and improved DNA quality. The standard CTAB method is commonly used for DNA extraction, but it can be time-consuming and result in low-quality DNA due to the presence of contaminants such as polysaccharides, polyphenols, and RNA. The modified protocol also yielded higher DNA amounts than the standard CTAB method and produced DNA of higher purity. This was evident from the 260/280 ratio of approximately 1.8, indicating that the extracted DNA was free from protein contamination. The results have significant implications for genetic studies of mango and other plant species. The use of 0.4 M glucose helped to remove major contaminants and polysaccharides, resulting in high-quality DNA extraction from both young and mature mango tissues. This method can be adopted in future studies of mango and other plant species to obtain high-quality DNA for accurate genetic analysis.

Akbar *et al*. (2014) conducted a study to assess the genetic diversity of mango varieties in Pakistan using 14 SSR markers revealed a high degree of genetic diversity among the varieties. They identified a total of 76 alleles, with an average of 5.4 alleles per locus, indicating a high level of genetic variability among the mango varieties. They used clustering and principal coordinate analysis to determine the genetic relationships among the mango varieties. The analysis showed that the mango varieties could be divided into two major groups, indicating genetic differentiation among the varieties.

Haque *et al.*(2015) conducted the genetic diversity of 18 mango cultivars in Bangladesh using 12 ISSR markers and recorded high level of genetic diversity among the mango cultivars, with the ISSR markers being effective in differentiating between closely related cultivars. They identified a total of 145 ISSR markers, of which 138 were polymorphic, indicating a high degree of genetic diversity in the mango cultivars and suggested that ISSR markers can be a useful tool for assessing the genetic variability of

mango germplasm in Bangladesh used clustering and principal coordinate analysis to determine the genetic relationships among the mango cultivars.

Saleem *et al*. (2018) investigated the genetic diversity of 23 mango varieties in Punjab, Pakistan, using SSR 15 markers showed a high level of genetic diversity among the mango varieties, with a total of 111 alleles detected across all the markers. The average number of alleles per marker was 7.4, indicating high genetic variability. They calculated various genetic diversity parameters, including observed and expected heterozygosity, polymorphism information content (PIC), and genetic similarity. The observed heterozygosity ranged from 0.095 to 0.826, with an average value of 0.406. The expected heterozygosity ranged from 0.158 to 0.873, with an average value of 0.686. The PIC values ranged from 0.152 to 0.841, with an average value of 0.636, indicating the high informativeness of the markers used. The genetic similarity analysis showed that the mango varieties were divided into two main clusters, and principal coordinate analysis (PCoA) accounted for 38.45% of the total variation.

Sarika *et al*. (2019) assessed the genetic diversity of mango 51 varieties in Kerala, India, using 20 SSR markers resulted with high level of genetic diversity among the mango varieties, with a total of 184 alleles identified, of which 179 were polymorphic. The average number of alleles per locus was 9.2, indicating a high level of genetic variation. They showed a low level of genetic differentiation among the mango varieties, suggesting that the mango varieties in Kerala were closely related. The clustering analysis revealed a clear separation of the mango varieties into three major groups, based on their geographical origin.

Hadhary *et al*. (2015) evaluated the genetic diversity of 103 wild mango varieties from different regions of India using 18 SSR markers. The high level of genetic diversity among the wild mango varieties. The observed heterozygosity ranged from 0.370 to 0.959, with an average of 0.703. The expected heterozygosity ranged from 0.397 to 0.939, with an average of 0.718. It was found that the wild mango populations were structured into three major groups based on their geographical origin. The region-specific conservation efforts and identifies potential sources of genetic material for the improvement of cultivated mango varieties. The other markers such as single nucleotide polymorphisms (SNPs) or whole-genome sequencing to can be used.

Ariffin *et al*. (2015) evaluated the genetic diversity and relationships of *Mangifera* species and cultivars/landraces/clones using inter simple sequence repeats (ISSR) markers in Malaysia and assessed the level of genetic variation among 28 accessions of *Mangifera* species, including wild relatives, landraces, and clonal varieties. They generated 198 markers

using nine anchored primers and one non-anchored primer. They found that the average degree of polymorphism was 98%, indicating a high level of genetic variation among the accessions of *Mangifera* species and the genetic diversity among 18 accessions of *M. indica*, and found that the Dice index of genetic similarity ranged from 0.2588 to 0.7742. The dendrogram showed that the 18 accessions of *M. indica* were grouped into three main clusters. Cluster I comprised 10 landraces of *M. indica* from Kedah, Cluster II comprised 7 landraces of *M. indica*, and Cluster III comprised Chokanan.

Bhattarai *et al.*(2016) evaluated the genetic diversity of 70 mango landraces in the eastern Himalayan region of India using 10 microsatellite markers, which showed a high level of genetic diversity, with 204 alleles detected across the 10 microsatellite markers. The average number of alleles per locus was 20.4, indicating a high level of genetic variability with an average polymorphism information content (PIC) value of 0.76. The mango landraces were genetically distinct, indicating the importance of preserving them for their unique genetic traits. The genetic distance analysis showed that the landraces were grouped into four major clusters based on their geographic origin. The significant correlation between geographic distance and genetic distance, indicating the presence of genetic differentiation among mango populations in the region.

Maina *et al*. (2016) conducted a study on the genetic diversity of 77 mango cultivars in Kenya using 12 microsatellite markers to understasnd the genetic diversity and relationship among the mango cultivars and to identify unique genetic traits for their conservation and breeding programs. They found high level of genetic diversity among the cultivars, with 117 alleles detected across the 12 microsatellite markers. The average number of alleles per locus was 9.75, indicating a high level of genetic variability. It revealed a high level of polymorphism, with an average polymorphism information content (PIC) value of 0.69.

Joshi *et al.*(2016) evaluated genetic diversity of 60 mango varieties grown in Karnataka using SSR techniques. The principal coordinate analysis (PCoA) and unweighted pair-group method with arithmetic mean (UPGMA) were used to analyze the genetic relationships among the mango varieties. The molecular data with the morphological characteristics of the mango varieties were studied. They found that relying solely on morphological traits may not be sufficient to differentiate between different varieties of mangoes.

Ashfaq *et al.* (2017) evaluated the genetic diversity of 60 wild mango varieties from 14 different regions of Pakistan using SSR (Simple Sequence Repeat) markers. The PCR amplification of genomic DNA using SSR primers produced a total of 114 bands, out of

which 108 were polymorphic, indicating a high level of genetic diversity among the wild mango varieties from Pakistan using statistical methods to analyze the data, including cluster analysis, genetic diversity analysis, and principal coordinate analysis (PCoA).Cluster analysis based on the genetic similarity coefficients revealed four distinct clusters, with some samples showing a high degree of genetic similarity within the clusters. The genetic diversity analysis showed that the observed heterozygosity ranged from 0.04 to 0.67, and the expected heterozygosity ranged from 0.16 to 0.71, indicating a wide range of genetic diversity among the wild mango varieties from Pakistan. The PCoA results showed that the first two principal coordinates accounted for 51.6% of the total variation, indicating a high level of genetic diversity among the samples.

Ng *et al*.(2017) conducted a study to genetic diversity of wild mango populations in Malaysia using 12 microsatellite markers to determine the level of genetic diversity, population structure, and gene flow among the wild mango populations. A moderate level of genetic diversity among the populations, with an average observed heterozygosity of 0.49 and an average expected heterozygosity of 0.67. It was revealed a low level of inbreeding within the populations, indicating a healthy level of genetic variability. The population structure analysis revealed that the wild mango populations in Malaysia can be grouped into three distinct genetic clusters, indicating the presence of genetic differentiation among the populations.

Paliwal *et al.* (2018) evaluated the genetic diversity of 39 wild mango varieties from various regions of India using 25 SSR markers. The PCR amplification of genomic DNA using SSR primers produced a total of 205 bands, out of which 182 were polymorphic, indicating a high level of genetic diversity among the wild mango varieties from India. Cluster analysis based on the genetic similarity coefficients revealed four distinct clusters, with some samples showing a high degree of genetic similarity within the clusters. The genetic diversity analysis showed that the observed heterozygosity ranged from 0.25 to 0.85, and the expected heterozygosity ranged from 0.26 to 0.86, indicating a wide range of genetic diversity among the wild mango varieties from India. The PCoA results showed that the first two principal coordinates accounted for 39.1% of the total variation, indicating a moderate level of genetic diversity among the samples.

Singh *et al*. (2018) studied genetic diversity of 17 wild mango varieties from Uttarakhand, India, using 16 SSR (Simple Sequence Repeat) markers. The SSR markers are highly polymorphic and have the advantage of being co-dominant, which allows for the identification of heterozygotes. The PCR amplification to amplify the DNA of the mango

samples and analyzed the resulting banding patterns.The results showed a high level of genetic diversity among the wild mango varieties. They detected a total of 146 alleles across all the samples, with an average of 9.1 alleles per locus. The percentage of polymorphic loci was 94.44%, indicating a high level of genetic variation. The genetic diversity index (He) among the samples ranged from 0.408 to 0.808, with an average of 0.614, indicating moderate to high genetic diversity among the samples. They performed cluster analysis to group the samples based on their genetic similarity. The dendrogram showed four distinct clusters with some samples showing a degree of genetic similarity within the clusters.

Patil *et al*. (2019) determined the molecular profile of Alphonso mango from different locations in South Konkan using PCR and ISSR markers. The results showed a clear banding pattern in the PCR analysis, indicating a better and more reliable method for assessing genetic diversity in mango. The ISSR markers showed a similarity range of 0.559 to 0.733, with an average PIC value of 0.733 per primer. The genetic variation was present in 10 different locations and within 5 individual samples. This indicates that Alphonso mango exhibits a high degree of genetic diversity, even within a single location. It can be used to identify the distinctiveness of the variety in diverse geographical locations. They assessed 19 different genotypes of mango using 20 simple sequence repeat markers and grouped them into two clusters. Pusa Surya was found to be the most diverse genotype, both morphologically and genetically. The analysis of vegetative growth characteristics of different genotypes of mango was also conducted.

Sarika *et al*.(2019) evaluated the genetic diversity of 51 mango varieties in Kerala, India, using 20 SSR markers. They revealed a high level of genetic diversity among the mango varieties, with a total of 184 alleles identified, of which 179 were polymorphic. The average number of alleles per locus was 9.2, indicating a high level of genetic variation. It was showed a low level of genetic differentiation among the mango varieties, suggesting that the mango varieties in Kerala were closely related. The clustering analysis revealed a clear separation of the mango varieties into three major groups based on their geographical origin.

González-Arnao *et al*. (2019) evaluated the genetic diversity of 23 mango cultivars in Mexico using both morphological and molecular markers for 14 quantitative and qualitative traits, including fruit shape, fruit weight, and tree height. The analysis revealed significant differences in the morphological traits among the cultivars and 22 SSR markers. The molecular analysis revealed a high level of genetic diversity among the cultivars, with an average of 9.4 alleles per locus. It was identified three distinct groups of mango cultivars

based on their genetic similarity. The combination of morphological and molecular markers helped to obtain a detailed assessment of the genetic diversity of mango cultivars in Mexico.

Kannan *et al*. (2019) assessed the genetic diversity of 58 mango cultivars in India using both morphological and 20 SSR markers. They assessed 16 quantitative and qualitative traits, including fruit weight, fruit shape, and flesh color. The analysis revealed significant differences in the morphological traits among the cultivars. The molecular analysis revealed a high level of genetic diversity among the cultivars, with an average of 7.2 alleles per locus. The four distinct groups of mango cultivars based on their genetic similarity. The genetic diversity of Indian mango cultivars is rich and diverse, which provides an excellent opportunity for breeding programs to select desirable traits.

Singh *et al*. (2019) conducted a study on the morphological and genetic diversity of mango cultivars in India. They sequenced the RNA from the leaves of three mango cultivars, 'Neelam', 'Dashehari', and their mixture 'Ambrapali', and identified 1.67 million high-quality SNPs across 84 mango cultivars. It was included the sequencing of 323 Mb of the Heterozygous Ambrapali Genome and the identification of 43,247 predicted protein-coding genes. The morphological parameters such as leaf size, leaf shape, shoot length, fruit size, fruit shape, stone size, and stone weight were also evaluated in this study and used SSR amplification polymerase chain reaction (PCR) with 109 microsatellite markers to assess the genetic diversity within the entire sample. The gel dock system was used to analyze the products separated by agarose gel electrophoresis using Tris-acetate EDTA (TAE) buffer at pH 8. They identified a total of 198 alleles to study the genetic diversity of the mango cultivars. It provided valuable insights into the morphological and genetic diversity of mango cultivars in India. The use of RNA sequencing and SNP markers allowed for the identification of high-quality genetic markers, which could be used in future studies for cultivar identification, breeding, and conservation

Yamanka *et al*. (2019) assessed the genetic diversity and relatedness of 120 mango (*Mangifera indica* L.) genetic resources in Japan using 46 simple sequence repeat (SSR) markers. They distinguished 83 genotypes using 10 sets of three SSR markers, except for synonymous and identical accessions. Parentage analysis was carried out and both parents of 11 accessions were newly identified or reconfirmed. Over 30 cultivars were found to be offspring of 'Haden'. Genetic relatedness and diversity analyzes revealed three distinct clusters, two of which corresponded to the groups of USA and India. The third cluster included accessions from Southeast and East Asia. The Florida accessions, which originated

from hybrids between these two types, were found to be more closely related to the Indian type.

Srivastava *et al.* (2021) studied the genetic diversity of 24 mango genotypes using SSR markers. The SSR markers were highly variable, with a range from 0.10 to 0.78 for polymorphism, and a mean PIC value of 0.40. The highest value was observed for MSSR133. The genotypes were grouped into two clusters based on their origin, parentage, and embryo types using UPGMA analysis. The UPGMA analysis revealed two clusters of genotypes. By understanding the genetic diversity of mango genotypes, one can identify those with desirable traits and develop strategies to improve crop yields and quality.

Gayathri *et al.* (2021) evaluated the development of 34 SSR markers in 36 Okra (*Abelmoschus esculentus*). RNA from various organs of okra was used to generate complete transcriptome data, and a total of 10,492 SSRs were identified, with tri-repeats being the most common repeat motif. Among them 18 SSR primers were highly polymorphic, indicating their usefulness for germplasm characterization, mapping, genetic diversity studies, molecular assisted breeding, and gene discovery in okra. The expected heterozygosity values ranged from 0.125 to 0.971 with a mean value of 0.593, while the observed heterozygosity values ranged from 0.000 to 0.839 with a mean value of 0.203. The number of alleles per locus ranged from 1 to 30, and the polymorphic information content (PIC) ranged from 0.119 to 0.955, with a mean PIC value of 0.554. Probability of identity (PI) values ranged from 0.0036 to 1.0000 with a mean value of 0.263.

Sharma *et al*. (2021) evaluated the genetic diversity and structure of mango cultivars in the Caribbean using 14 SSR microsatellite markers. The three main genetic clusters identified in the study were consistent with the geographic origin of the cultivars. This suggests that there is a strong correlation between the genetic structure of mango cultivars and their geographic origin.

Hasan *et al*. (2022) assessed the genetic diversity of 38 mango cultivars from Bangladesh using SSR markers. They collected young leaves from the cultivars and extracted genomic DNA, which was used to amplify 20 SSR loci. The results showed that all 20 SSR loci were polymorphic, with a total of 116 alleles detected across the cultivars. The mean number of alleles per locus was 5.8, and the mean observed and expected heterozygosity were 0.38 and 0.61, respectively. They used principal coordinate analysis (PCoA) and unweighted pair-group method with arithmetic means (UPGMA) clustering to evaluate the genetic relationships among the mango cultivars. It was showed that the cultivars could be grouped into two main clusters, with a moderate level of genetic diversity observed within

and between the clusters. The moderate level of genetic diversity as the prevalence of vegetative propagation and the widespread cultivation of a few popular cultivars in Bangladesh.

Wang *et al*. (2022) evaluated the genetic diversity of mango cultivars from different geographical regions using microsatellite markers, and they extracted DNA from young leaves of the mango cultivars and amplified 10 SSR markers to assess the genetic diversity. The results showed a high level of genetic diversity among the 49 mango cultivars, with a total of 83 alleles detected across the 10 SSR markers it was found an average of 8.3 alleles per locus, which indicates a high level of genetic diversity among the mango cultivars. The observed and expected heterozygosity values were also high, indicating that the mango cultivars have high levels of genetic variation and UPGMA clustering analysis to evaluate the genetic relationships among the mango cultivars. The mango cultivars were divided into four major clusters and the clustering of the mango cultivars was not correlated with their geographical origin, suggesting that the genetic diversity of mango cultivars is shaped by factors beyond geographic location.

Hong *et al*. (2023) studied the mango genetic diversity using SSR molecular markers to identify, characterize, and distinguish the genetic relationships among mango cultivars, providing critical insights into mango germplasm resources. The statistical analysis of SSR loci within the mango genome reveals intriguing patterns, with dinucleotide repeat elements being the most abundant, accounting for 40% of total SSR loci. This information is essential for understanding the genomic composition of mango varieties. The predominant nucleotide elements, repetition times, and lengths of SSR loci are highlighted, offering valuable data for marker development and genetic characterization. The exploration of the genetic diversity and phylogenetic relationships among 71 mango cultivars. The utilization of SSR markers and polymorphic primers enables the classification of these cultivars into distinct categories, providing a comprehensive overview of genetic relationships within the mango germplasm.

2.3 DNA Isolation and Quantification

Meyer *et al*. (2004) investigated the impact of different similarity coefficients used in cluster analysis on the outcomes of genetic diversity studies of maize using AFLP and RAPD markers used 18 inbred lines of maize from the BR-105 and BR-106 populations and calculated eight similarity coefficients including Jaccard, Sorensen-Dice, Anderberg, Ochiai, Simple-matching, Rogers and Tanimoto, Ochiai II, and Russell and Rao. The comparison of the similarity matrices was done using various statistical methods including Spearman correlation, cluster analysis with dendrograms, Tocher optimization procedure, and projection

efficiency in a two-dimensional space. It was indicated that Jaccard, Sorensen-Dice, Anderberg, and Ochiai coefficients exhibited comparable results for nearly all techniques and marker systems. The Simple Matching, Rogers and Tanimoto, and Ochiai II coefficients showed no significant changes in the results as they take into account negative co-occurrences. The Russell and Rao coefficient produced significantly different results from the other coefficients due to the exclusion of negative co-occurrences from the numerator but inclusion in the denominator. This demonstrated that the choice of similarity coefficient can significantly affect the outcome of cluster analysis in genetic diversity studies.

Bbosa *et al*. (2007) focused on investigated the antibacterial activity of leaf extracts from *Mangifera indica* L., on *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* using both the agar-well diffusion and gradient serial dilution methods. It was indicated that the extracts possessed antibacterial activity against all three organisms tested. Chemical tests were also conducted on the leaf extracts to identify potential bioactive compounds. The ether fraction contained saponins, steroids and triterpenoids, while the ethanolic portion contained alkaloids, anthracenocides, coumarins, flavonones, reducing sugars, catechol and gallic tannins, saponins, steroids, and triterpenoids. The aqueous fraction contained anthracenocides and flavonones, as well as reducing sugars. These results suggest that the antibacterial properties of *M. indica* leaf extracts may be due to the presence of these bioactive compounds.

Kit *et al.* (2010) isolated DNA from Chokanan mango leaves for the construction of a total genomic library. The quality of DNA was improved by removing secondary metabolites from the leaf using cetyl trimethylammonium bromide (CTAB), polyvinylpyrrolidone (PVP) and β-mercaptoethanol. The presence of polyphenolic compounds was removed using CTAB, while PVP and β-mercaptoethanol were used to eliminate the effect of reducing sugars and endogenous proteases. The high salt washing step was used to remove polysaccharides that can bind with the DNA. The extracted DNA was treated with RNase and purified using phenol-chloroform extraction and ethanol precipitation. The quality of the extracted DNA was analyzed using agarose gel electrophoresis and the purity was assessed by spectrophotometry. It was showed that the DNA yield was 221.3 μg per gram of fresh weight, with a purity ratio (A260/A280) of 1.86. The isolated DNA was further used for the construction of a total genomic library. The use of a high salt washing step effectively removed polysaccharides, which can interfere with the purity and integrity of the DNA.

Jagarlamudis *et al.* (2010) evaluated on molecular identification of mango, (*Mangifera indica* L.) var. totupura, using DNA extraction and sequencing techniques.

Tender leaves were used for DNA extraction by MATAB (methyl ammonium bromide) method. The extracted DNA was then amplified using PCR with chloroplast trnL-F primers. The sequencing of the amplified DNA was performed using the ABI PRISM Dye Terminator cycle sequence ready reaction kit, and the phylogenetic placement was done at the Ohmlina center for molecular research in Chennai. Leaves were ground in liquid nitrogen and adding extraction buffer followed by incubation at 74^0C for 30 min. The DNA was then precipitated and strained using agarose gel with ethidium bromide. The PCR amplification using the trnL-F primers was successful, and the resulting sequences were used to construct a phylogenetic tree for the *Mangifera* genus. They found that the MATAB method was effective in extracting DNA from fresh and tender leaves of the mango plant, and the PCR amplification using chloroplast trnL-F primers was successful in obtaining DNA sequences. The phylogenetic tree constructed using the sequences showed that *Mangifera indica* L. var. Totupura belongs to the *Mangifera* clade. The phylogenetic analysis of the resulting DNA sequences provided valuable information on the placement of *Mangifera indica* L. var. totupura in the *Mangifera* genus.

Azmat *et al.* (2012) conducted a study on the extraction of DNA suitable for PCR applications from mature leaves of *Mangifera indica* L. The DNA extraction protocol required a modified version of CTAB (Cetyltrimethylammonium bromide), and high concentrations of salt were used to remove polysaccharides. Polyvinylpyrrolidone (PVP) and β-mercaptoethanol were used to remove phenolic compounds. It showed that the extended chloroform-isoamyl alcohol treatment followed by RNase treatment yielded 950–1050 µg of good quality DNA. The extracted DNA was used for PCR applications using simple sequence repeat (SSR) primers. It was demonstrated that the modified CTAB protocol was effective for extracting good quality DNA from mature leaves of *Mangifera indica* L. The high concentrations of salt and additional treatments with PVP and β-mercaptoethanol were effective in removing polysaccharides and phenolic compounds, respectively. The extracted DNA was suitable for PCR applications using SSR primers.

Majumderi *et al.* (2013) evaluated the genetic divergence among 60 mango genotypes using morphological traits and the mango genotypes were grouped into 8 clusters based on the similarity of their morphological characteristics. The genetic divergence among the mango genotypes was mainly influenced by their morphology and not by their geographical distribution. The inter-cluster distance was found to be maximum in cluster II and cluster VIII, whereas it was lower in Cluster VII and Cluster VIII. Cluster I was found to be high yielding and ranked first in terms of number of secondary branches per inflorescence, percent

fruit set per inflorescence, and yield per plant. The Cluster VIII had only one genotype which produced the highest percentage of flowering shoots, % perfect flowers, number of fruits per plant, and % TSS. The genotypes of Cluster VII produced the largest sized fruits. The first nine characters of the principal component axes with eigen values above unity accounted for 88.3% of the total variation among the fifteen characters. Weight of harvested fruits per plant, number of fruits per plant, and individual fruit weight were identified as the important components of genetic divergence. The genotypes belonging to clusters I, VII and VIII with high to moderate genetic distances might be used as potential parents for hybridization and genetic improvement programs.

Begum *et al.* (2013) evaluated the molecular analysis of intracultivar polymorphism of 'Panchadarakalasa' mango using microsatellite markers. It involved the collection of fruit and leaf samples from 16 trees of 'Panchadarakalasa' from three eco-geographical regions of Coastal Andhra, Rayalaseema and Telangana of the state during summer 2009. The phenotypic variations among accessions under study based on 9 quantitative and 7 qualitative traits. Out of 109 mango-specific microsatellite markers validated, 20 were amplified, and only 4 were polymorphic with a total of 11 alleles ranging from 130 bp to 245 bp. The polymorphic information content of the polymorphic alleles ranged from 0.25-0.56. The dendrogram indicated that accessions were not grouped as per geographic separation. Microsatellite analysis revealed smaller intracultivar variability of 10% in in-situ conditions, and a genetic divergence between trees attesting that 'Panchadarakalasa' is not a pure clone, and that whatever is cultivated throughout the state is not genetically identical. DNA extraction from leaf samples was carried out using standard protocols, and the leaves were stored at -80^0C until the time of extraction.

Sharma *et al.* (2014) compared two different methods for extracting genomic DNA from mango leaf samples for next-generation sequencing. They collected leaf samples from 48 different mango varieties and used the CTAB method as described by Doyle and Doyle (1987) for DNA extraction. The extracted DNA was quantified and analyzed for quality. The authors reported that the CTAB method yielded higher amounts of DNA (1375.0ng/µL) with good quality (A260/230 1.80 and 1.90, respectively) suitable for downstream applications such as PCR, PacBio sequencing, ddRAD sequencing, and long-term storage. They evaluated the quality of the DNA extracted using the CTAB method by running PCR amplification of four different mango genes. The amplified products were sequenced and the results showed that the extracted DNA was of high quality and suitable for further sequencing applications. The importance of choosing the right DNA extraction method for downstream applications. It

was provided important insights into the advantages of using the CTAB method for genomic DNA extraction from mango leaves, which could be useful for other researchers working with mango or other plant species.

Toili *et al.* (2016) assessed the diversity of mango germplasm from the Upper Athi River (UAR) region in eastern Kenya to identify different mango varieties in the absence of fruits by using morphological traits. They selected 20 traits from the International Plant Genetic Resources Institute (IPGRI) descriptors for mango, and collected data from 98 mango accessions in 15 sites of the UAR region. The collected data were subjected to multivariate analysis, including Principal Component Analysis (PCA) and Cluster analysis, as well as one-way analysis of variance (ANOVA) and Chi-square tests. It was showed that the first six principal components accounted for 75.12% of the total variance, indicating significant diversity in mango germplasm in the UAR. It was revealed a strong and highly significant correlation between the color of fully grown leaves, leaf blade width, leaf blade length, and petiole length, as well as between leaf attitude, color of young leaf, stem circumference, tree height, leaf margin, growth habit and fragrance.

Vieccelli *et al.* (2016) studied the leaves and fruits of mango plants from cv. Imbú, Brazil to evaluate various descriptors related to leaf morphology, including leaf blade division, composition, petiole length, phyllotaxis, shape, margin, and texture, as well as fruit traits, including flesh color, weight, percentage of seeds, peel and pulp color, and cerosity. The intensity of green color on the leaf blade was found to be intermediate, with less intense staining on the abaxial surface compared to the adaxial surface. They showed that cv. Imbú did not have most of the desirable characteristics for a mango fruit that is usable in industrial processes or for fresh consumption, indicating that it is more suited as a rootstock.

Lauricella *et al.* (2017) studied on multifaceted health benefits of *Mangifera indica* L. The Inestimable Value of Orchards Recently Planted in Sicilian Rural Areas. The cultivation widely seen in areas of India, Africa, Asia, and Central America. Fleshy stone fruits rich in phytochemicals with high content of polyphenolics and vitamins. They provided the information on the antioxidant, anti-inflammatory, and anticancer properties of mango. A better characterization of phytocompounds found in Sicilian mango in comparison to those of tropical areas, as well as a broader analysis of their properties, could improve our knowledge of their biochemical activity and achieve the production of phytopharmaceuticals to associate with the most common therapies for some human disease treatments.

Nazish *et al*. (2017) studied the molecular diversity of 15 Pakistani indigenous mango (*Mangifera indica* L.) varieties using 12 microsatellite markers and 10 were found to

be polymorphic, while the remaining two were monomorphic. The dendrogram analysis showed coefficients of similarity ranging from 75 to 100%, indicating the frequent use of only a few parent cultivars and the presence of inbreeding. The identification of inbreeding among these cultivars highlights the need for improved breeding strategies to enhance genetic diversity and maintain the long-term sustainability of mango cultivation in Pakistan.

Thakur *et al.* (2017) studied the genetic variability of 10 mango cultivars using RAPD-PCR markers resulting in the observation of 99 amplicons, including two unique bands. The DNA isolation was carried out using a modified CTAB method, and amplification was performed using RAPD-PCR. The electrophoretic profile of the amplified DNA was analyzed for polymorphism based on the presence or absence of DNA bands on an agarose gel, and the sizes of DNA fragments were estimated by comparison with a standard ladder. The cluster analysis revealed a 27% to 64% similarity index among the mango cultivars, indicating a high level of genetic diversity. The importance of genetic diversity in the evolution of mango cultivars and suggests new possibilities for their future development. The high level of genetic diversity observed provide important genetic resources for breeding programs aimed at improving mango cultivars for traits such as disease resistance, fruit quality, and yield.

Igbari *et al.* (2018) evaluated the morphological features of 7 mango cultivars and determine their distinctiveness and diversity using different statistical tools. They collected from the National Horticultural Research Institute (NIHORT), Ibadan, Nigeria. The morphological features of these mango cultivars were assessed based on leaf length and width, leaf apex, petiole length, fruit length and width, fruit color, and shape. PCA, cluster, and dendrogram analyzes were performed to determine the distinctiveness and diversity of the mango cultivars. They found that mango cultivars were distinctly divided into two groups based on their fruit characteristics. Cluster A comprised Julie, Edward, Palmer, and Kent, while cluster B comprised Saigon, Madoe, and Lipen. Morphological characterization is an important tool for the assessment and management of genetic resources in plant breeding programs.

Kulkarni *et al.* (2018) extracted mangiferin from mango leaves for therapeutic applications and to identify the antidiabetic potentiality of mango leaves, which can be used in the therapy of diabetes mellitus. It was employed α-amylase inhibition assay using aqueous mango leaf extract and mangiferin compared with Acarbose. The α-glucosidase inhibition assay, which is present in the small intestine and helps balance blood sugar levels, was also performed. They reported that mangiferin has a significant inhibitory effect on α-amylase and

α-glucosidase, indicating its potential in the treatment of diabetes mellitus. The mangiferin extracted from mango leaves can be used in the cosmetic industry and maintained in purity conditions for the pharmaceutical industry. FTIR/ Fourier Transform Infrared Spectrum analysis was performed to determine the characteristics of mangiferin extracted from mango leaves.

Janowski *et al.* (2019) isolated as purified nucleic acids from the mango leaves. They have developed a simple, rapid, cost-effective, and reliable protocol for DNA isolation from cultured fungi on slants and from dried fungal samples using silica particles (silicon dioxide powder) in chaotropic conditions. Compared with other methods employing CTAB or commercial kits, it allows fast, easy, and cheap DNA purification from two main sources of fungi routinely used for research. One of the most demanding DNA extractions is from bones and teeth due to the robustness of the material and the relatively low DNA content. Our laboratory works with two different extraction methods based on silica membranes or the use of silica beads. They found that the DNA extraction using the silica membrane method results an in higher DNA.

Sridhar *et al.* (2019) studied 16 traditional mangoes using 26 morphological and 20 SSR markers. PCA shows 84.25% genetic diversity in cultivars. UPGMA dendrograms classified 16 mangoes into five major clusters. The dendrogram shows highest similarity 88% was observed in between Ranipasand and Gulab Khas. Isolation of DNA using modified CTAB and quality was checked in 1% agarose gel. There are three alleles per locus (3.38). The Jaccard's similarity coefficient values varied from 0.03 to 0.88. The present finding of clustering of large-sized mango cultivars into single clusters. Cluster V had maximum intracluster D2 value indicating wide genetic variation among the cultivars. The variability observed among the cultivars due to cross pollination and evaluation shows high level of polymorphism helps to select the diverse parents in mango breeding programme. Cluster 4 includes Gulab Khas, Ranipasand and Chatterjee in cluster A, Madhu chuski and Khota lagga under cluster B, cluster C includes sarikhas, Banganpalli, langra, fazli, Himasagar. D and E comprises Gopal bhog, Kancha mithra and Kanchan kosha as well as Kamala bhog.

Song *et al.* (2023) studied on advances in sequencing and key character analysis of mango (*Mangifera indica* L.) Genomic sequencing technologies, including next-generation sequencing (NGS) and whole-genome sequencing (WGS), have played a pivotal role in uncovering the complex genetic makeup of mango. NGS has allowed for the rapid and cost-effective identification of genetic markers, such as Single Nucleotide Polymorphisms (SNPs) and Simple Sequence Repeats (SSRs), enabling high-resolution genetic diversity studies,

germplasm characterization, and marker-assisted breeding programs. WGS has provided comprehensive insights into the mango genome, offering a foundation for understanding gene function, trait mapping, and potential genetic improvements. Key character analysis has also advanced our understanding of mango traits critical for breeding and commercial production. This traits related to fruit quality (sugar content, acidity, aroma), disease resistance, fruiting behavior, and adaptability to diverse environmental conditions were included.

3.0 MATERIALS AND METHODS

3.1 Materials

3.1.1 Plant Materials

All together 45 Mango varieties of Dakshina Kannada and Udupi Districts were used in the present investigation for morphological and molecular characterization using SSR markers. These wild varieties of mango belong to the different locations of Dakshina Kannada and Udupi.

3.2 Methodology

Using Biodiversity International descriptors, morphological characterization of the 45 selected wild mango varieties from different places of Dakshina Kannada and Udupi districts was carried out for fruits (IPGRI 2006).

3.2.1 Morphological Diversity

The morphometric traits of 45 mango varieties were carried out using biodiversity international descriptors (IPGRI 2006). The sampling of wild mangoes was collected during in the month of April-May 2019, following simple random strategy the fresh young leaves and ripe fruits of wild *Mangifera indica* were collected from different locations of Dakshina Kannada and Udupi districts of Karnataka with Global Positioning System. The investigation of wild mangoes in the regions of Dakshina Kannada at latitude 12°48'36" and longitude 75°06'22", Bantwal to Moodbidri, and coastal Karnataka at latitude 13°26'09" and longitude 75°01'56", Karkala to Hebri. The evaluation of genetic diversity of wild mangoes was carried out at the Division of Basic Sciences, ICAR-Indian Institute of Horticultural Research, Hesaraghatta, Bangalore during 2019-20.

The observations were recorded on plants chosen at randomly in each variety of Dakshina Kannada and Udupi districts of Karnataka. Data were recorded on the basis of quantitative and qualitative characters. The average values were computed as treatment mean under each replication. The characters studied and techniques adopted to record the observations. There are 14 Quantitative characters such as leaf blade length, leaf blade width, petiole length, fruit length, fruit diameter, fresh fruit weight, fruit width, pulp percentage, Total Soluble Solid, peel percentage, fruit thickness (By using vernier caliper), stone length, stone thickness, stone weight and 11 Qualitative characters leaf apex shape, leaf base shape, leaf fragrance, skin colour, pulp colour, shape of fruit apex, skin thickness, height of the tree, fruit shape, fruit beak type, crown shape were recorded.

3.2.1.1 Leaf Sampling

Mature leaves of each of the sampled wild varieties were collected for morphological genetic characterization. It was kept in a freezer at -80^{o}C. Using a vernier calliper, the length in cm from the base of the leaf to the end of the leaf blade was measured. After that, the width, petiole, and form of the leaf were also assessed visually. Mature leaves of each of the variety were collected for the morphometric chracterization. Leaf blade shapes are elliptic, oblong, ovate, obovate, lanceolate, oblanceolate. Leaf blade length measures in cm and average 10 mature leaves measured from the base to the tip of the leaf blade. Leaf blade width measures in cm and average of 10 mature leaves measured at the widest point, Leaf apex shapes are obtuse, acute, acuminate. Leaf base shapes are acute, obtuse, round. Leaf fragrance was recorded for mature leaf like absent/mild/strong.

3.2.1.2 Fruit Sampling

Ten mango fruits were taken from each wild cultivator in a simple random selection. Morpho-physiological character of fruit sample were recorded following descriptors of mango (IPGRI 2006). The samples were examined for quantitative traits like fruit length (cm), fruit width (cm), fruit thickness (cm), fruit weight (gm), fiber length (mm), peel (%), pulp (%), stone weight (g), TSS (Brix), and qualitative trades like fruit shape, skin color, skin thickness, skin texture, quantity of fiber, pulp color, and eating quality. (Begum *et al.* 2013). In fruit length (cm), fruit diameter, fruit weight were recorded for the average of 10 fruits measured for morphometric analysis incuding with pulp percentage and peel percentage. Fruit shapes and shape of the fruit apex were recorded such as oblong, elliptic, roundish, ovoid, obovoid and acute, obtuse, round respectively. Fruit skin thickness was measured in mm for average of 10 ripe fruits and also skin and pulp colour. Fruit beak type were measured in Perceptible, pointed, prominent. Stone length, stone weight, stone thickness were measured for 10 fruits.

3.2.1.3 Fruit Colour

Fruit colour variations included greenish yellow, pale yellow, yellowish orange and yellowish green.

3.2.1.4 Fruit Weight (g/fruit)

The weight of a single fruit was measured and stated in grams; the weight of 10 fruits in each varieties was also measured, and the mean of these two values was reported as the mean fruit weight across all 45 varieties.

3.2.1.5 Fruit Width (cm)

Fruit width was measured and represented in centimetres for 10 random fruits across all varieties.

3.2.1.6 Tree Growth Samplings.

The growth habit of the tree was assessed with (IPGRI 2006), comparing them with Figureures followed by classification vertical, semi vertical and open (Ribeiro *et al.* 2013).

3.2.1.7 TSS or Total Soluble Solids

Using an Erma hand refractometer (0-32 Brix), the juice from five randomly chosen fruits from all varieties was squeezed to determine the amount of total soluble solids present.

3.3 Morphological Data Analysis

3.3.1Analysis of Variance (ANOVA)

To identify the variability, analysis of variance (ANOVA) was performed on all quantitative characteristics. Among the 45 mango varieties, the variances were examined in accordance with the norm. Gomez & Gomez (1984) proposed a technique suitable to randomized block design. ANOVA (Analysis of Variance) is a powerful statistical technique used to compare means between three or more groups, assessing whether there are significant differences among them. It examines the variability within groups and compares it to the variability between groups, determining whether the differences observed are likely due to genuine effects or simply random variation developed by Sir Ronald Fisher in the early 20th century, ANOVA has become a cornerstone in experimental design and hypothesis testing. Fisher's seminal work, particularly his book "The Design of Experiments" published in 1935, laid the foundation for ANOVA and revolutionized the field of statistics.

ANOVA works by partitioning the total variance observed in a dataset into different components: the variance due to differences between groups (treatment effect) and the variance due to differences within groups (error or residual variance). By comparing these variances through the F-test, ANOVA determines whether the differences between group means are statistically significant. There are several types of ANOVA depending on the experimental design, including one-way ANOVA for a single factor, factorial ANOVA for multiple factors, and repeated measures ANOVA for within-subject designs. Each type has specific assumptions regarding the data, such as normality and homogeneity of variances, which should be met for the results to be valid.

ANOVA has widespread applications across various disciplines, including psychology, biology, economics, and engineering. Researchers use ANOVA to investigate the effects of different treatments, interventions, or factors on outcomes of interest. It allows

researchers to draw conclusions about the population based on sample data, providing insights into the relationships between variables and informing decision-making processes. It is a fundamental statistical tool for comparing means across multiple groups, with roots in the pioneering work of Ronald Fisher, whose contributions continue to shape modern statistical methodology.

For the analysis of variance, the genotype mean values from each replication were employed. Using the mean values of each plot, the approach described by Panse & Sukhatme (1967) was followed to conduct the analysis of variance and covariance for each individual character and for character pairs, respectively. Using the error variance and the F-test, the significance of the differences between all genotypes were evaluated.

3.3.2 Variability studies

3.3.2.1 Phenotypic and Genotypic Variance (Lush (1949); Choudhary & Prasad 1958)

Genotypic Varaince$\left(\sigma_g^2\right) = \frac{\text{TMSS}-\text{EMSS}}{\text{r}}$

Error of variance$\sigma_e^2 = \text{EMSS}$

Phenotypic variance $\sigma_p^2 = \sigma_g^2 + \sigma_p^2$

where,

TMSS= Mean sum of square due to genotypes

EMSS= Mean sum of square due to error

σ_g^2= Genotypic variance

σ_e^2= Error variance

r = Number of replications

3.3.2.2 Phenotypic and Genotypic Coefficient of Variation (Burton & DeVane 1953).

Genotypic and phenotypic coefficients of variation (PCV) were given below**,**

PCV= $\frac{\sigma_p}{\bar{X}} \times 100$ GCV = $\frac{\sigma_g}{\bar{X}} \times 100$

where,

σ_p = Phenotypic standard deviation

σ_g = Genotypic standard deviation

$\bar{X}$= Grand mean.

PCV and GCV were classified as given below(Sivasubramanian & Menon 1973).

0-10% : Low

10-20% : Medium

20%and above: High

3.3.2.3 Heritability (Hanson *et al*. 1956)

Heritability (h^2)

$h^2 = \frac{\sigma_g^2}{\sigma_p^2} \times 100$ where

h^2= Heritability (broadsense)

σ_g^2= Genotypicvariance

σ_p^2= Phenotypicvariance

3.3.2.4 Genetic Advance (Johnson *et al*. 1955)

Genetic advance (GA)=$K \times \sigma_p \times h^2$

where,

K=Selection differential at 5percent selection intensity K=2.06 (Falconer 1994).

σ_p=Phenotypic standard deviation and

h^2=heritability (broad sense)

3.3.2.5 Genetic advance as percentage of mean(GAM) (Johnson *et al*. 1955)

By applying the formula, this was computed.

GA as per cent of mean = $\frac{GA}{Mean} \times 100$

The approach was used to classify the GA as a percentage of the mean as shown below.

0-10%	:	Low
10-20%	:	Medium
20 %and above	:	High

3.3.3 QGIS 3.36.3

The specific version of the open-source Geographic Information System (GIS) software called QGIS. QGIS is used for viewing, editing, and analyzing geospatial data. Here are some of the key features and updates you can expect in QGIS 3.36.3:

Key Features

User Interface (UI) Improvements:Improved usability and customization options for the user interface.Improved layouts for various tools and functions.

Data Handling:Better support for various geospatial data formats.Improved data management tools for handling large datasets.

Analysis Tools:Enhanced geoprocessing tools for spatial analysis.More robust and efficient algorithms for various spatial operations.

Display:Improved rendering capabilities for maps and layers. Advanced symbology options for better map presentations.

Plugins and Extensions:Compatibility with a wide range of plugins to extend the functionality of QGIS.Updates and bug fixes to existing plugins.

Integration:Better integration with other GIS and data analysis software. Enhanced support for web-based GIS services and data sources.

An investigation on "**Evaluation of Genetic variability of wild Mango varieties of Dakshina Kannada and Udupi Districts of Karnataka using morphological and molecular markers**" was conducted in the Division of Basic Sciences, ICAR-Indian Institute of Horticultural Research, Bangalore during 2019-2020.

At the site of the experiment, materials were utilized, laboratory procedures were performed, and molecular characterisation using SSR markers was done. Software was then used to analyze the findings, which are shown below in the following. a pair of groups.

1. To use SSR markers to examine the genetic diversity among mango wild varieties.

3.1Materials

3.1.1 Plant Material

In the current investigation, 42 wild mango varieties were employed. Young leaves were gathered in a various regions, including Hebri, Karkala, Moodbidri, Bantwal in the districts of Udupi and Dakshina Kannada. These samples were stored at -80°C in the ICAR-IIHR in Bengaluru before being ground into a fine powder under liquid nitrogen. During repeated washing steps using chloroform:isoamyl alcohol, whole genomic DNA was recovered using a modified CTAB technique (Ravishankar *et al*. 2000). Using a spectrophotometer (Gene Quanta, Amersham Bio-Sciences, Holliston, USA), the amount of DNA was measured by measuring absorbance at 260/280 and 260/230 nm.

3.2 DNA Extraction (Doyle, J. J., & Doyle, J. L. 1987)

3.2.1 Buffers and solutions prepared for extraction of DNA

Extraction buffer (10X): prepared using the following chemical constituents

CTAB (10%): 20g CTAB put on to make 200 mL final volume with sterile, twice-distilled water.

4 M NaCl: 82.9 g of NaCl dissolved in sterile twice distilled water and final volume put up to 100 ml.

1 M Tris-Cl (pH 8.0): 15.76 g of Tris-Cl added to sterile twice distilled water and the final volume make up to 100 mL. By using concentrated HCl, the pH was brought to 8.0.

0.5 M EDTA (pH 8.0): 14.612 g of EDTA dissolved in 100 mL double distilled water and pH brought to 8.0 by adding NaOH pellets.

From the above-mentioned stock solutions, 2% extraction buffer prepared as follows:

Table 1: The components of 2% extraction buffer

SI. No.	Components	Stock Concentration	Quantity	Final Concentration
1	CTAB	10%	200 mL	2%
2	NaCl	4.0 M	350 mL	1.4M
3	Tris-HCl	1.0 M	100 mL	20mM
4	EDTA	0.5 M	40 mL	100mM
5	Sterile Water	-	310 mL	-
6	**Total**		1000mL	

2% -Mercaptoethanol

Chloroform: Isoamyl alcohol prepared in the ratio of 24:1(v/v): Chloroform of 96mL mixed with 4mL of isoamyl alcohol and stored under refrigerated conditions.

5M NaCl- 292.2g of sodium chloride dissolved in 200mL of twice distilled water, final volume make to 300ml with distilled water.

Ethanol (70%) At room temperature, combine 70 mL of absolute alcohol with 30 mL of distilled water.

50X TAE

Table 2: Components for 50X TAE buffer

Sl. No.	Components	Molecular weight g/mol	Molarity in 50X	The amount for 1 l of 50X buffer
1	Tris base	121.14	2M	242g
2	Glacial acetic acid	60.05	1M	57.1ml
3	EDTA (pH 8.0)	372.24	50Mm	18.61g

242g of Tris-base dissolved in approximately 700mL of deionized water.

57.1 mL of 100 % glacial (acetic acid) and 100mL of 0.5 M EDTA (pH 8.0), added to beaker and final volume adjusted to 1liter.

3.2.2 Genomic DNA isolation from mango leaves

The efficacy and dependability of allelic diversity analysis and other molecular biology techniques depend entirely on pure and high molecular weight DNA, hence DNA extraction and purification are of utmost significance. Enzymatic processes might be slowed down by any DNA contaminants. Quantification and electrophoresis. Healthy, ideally young leaves were used to harvest DNA. Use the modified CTAB approach. First, ethanol was used to wipe the leaves clean.

Then, using an extraction buffer, leaves were pulverized in a mortar and pestle. seclusion from obtaining DNA from plant cells required just two simple processes.

1) Plant cells are lysed.

2) The removal of protein contaminants by the enzymatic or chemical solubilization of DNA.

3.2.3 Modified CTAB method used for DNA extraction

Ravishankar *et al*. (2013) described a modified CTAB approach for extracting DNA from leaves.

Procedure for DNA extraction from mango leaves

a. Two gram sample of the leaf tissue was homogenized in liquid nitrogen (-196ºC) using a mortar and pestle as part of the process for extracting DNA from mango leaves.
b. Following thorough mixing by gentle inversion, 10 ml of pre-warmed extraction buffer (containing 0.2% mercaptoethanol) was added to the crushed powder. A pinch of PVP and sodium acetate were also added. The mixture was then incubated at 65°C for one h in a water bath.
c. The sample was taken out of the heat and allowed to cool until it reached room temperature.
d. Adding isoamyl alcohol (24:1) to 10 mL of chloroform and mixing by inversion to create an emulsion.
e. Following complete mixing, it was centrifuged at room temperature for 15 min at 8000 rpm.
f. With the use of a wide-bore pipette tip, the aqueous phase was transferred to a fresh tube. To clear out the cloudiness in the aqueous phase, repeat the chloroform:isoamyl alcohol extraction step.
g. Two volumes of cold isopropanol were added and mixed by inversion after 1/2 volume of 5 M NaCl was added to the recovered aqueous solution.placed overnight in a freezer (-20°C) to precipitate.
h. Spun for 15 minutes at 5000 rpm after incubation. The particle was further rinsed with cold ethanol and finally the supernatant was poured off. Pellet dried in an oven for two minutes at 37°C. By flicking the tubes, the pellet was then dissolved in 1 mL of PCR water.
i. The DNA solution is mixed with 2 µL of RNase (10 mg/l) and incubated at 27°C in a water bath for 30 minutes to eliminate the RNA contamination.
j. DNA was lastly kept at -20°C till usage.

3.2.4 DNA extraction by Silica-coated magnetic beads (PuroMAG ™)

a. The genomic DNA was isolated by a protocol described by Li *etal*. (2010) with slight modification.
b. Young leaf tissue (100 mg) was taken in to 2 ml tube of round bottom and 500 µl of pre warmed CTAB buffer (2%) was added and leaf tissue was homogenized by bead beater (FastPrep-24™ Classic Instrument by MP Biomedical).
c. Tube containing homogenized samples were at 65°C in water bath for 15 min and gently mixed twice or thrice to increase the quality and yield of DNA.
d. The samples were removed and left to warm up on their own. Each tube was then filled with 500µL of the 24:1 chloroform:isoantyl alcohol solution.
e. At 4°C, tubes were centrifuged for 5 minutes at 12,500 rpm.
f. Using a micropipette, 200–300 µL of the clear aqueous supernatant was transferred to a new tube along with an equivalent amount of 6M sodium iodide (Nal) and 20–30 µL of silica dioxide (SiO_2).Tubes were gently vortexed and incubated for two h at room temperature.
g. Vortexed tubes were used and centrifuged for 2 min at 12500 rpm at room temperature and the supernatant was decanted leaving pellet at the base of tube.
h. 1ml washing solution was added to the pellet and vortexed.Tubes were centrifuged at 12500 rpm for 30 sec and supernatant was discarded.
i. The above step seven was repeated for second washing.
j. Micropippettes were used to gently remove the supernatant from the pellet, leaving it behind. The pellet was then allowed to air dry at room temperature.
k. 80-100 µl nuclease free water added to the pellet containing silica matrix.
l. Genomic DNA was eluted from silica matrix by vortexing the tube containing water and pellet by incubating the tubes at 70^0 C for 3-5 min.
m. The tubes were removed after three to five minutes and centrifuged at 12,500 rpm for two min.
n. DNA-containing clear supernatant was transferred to a new tube and kept at -20°C.
o. The genomic DNA was loaded an to 0.8% agarose gel in order to check the quality of it and Rnase(20ng/100 µl) was added if at all RNA contamination was there.

p. Concentration and quality were further examined using UV-spectrophotometer on Nabi UV/VIS Nano Spectrophotometer Ver.3.6.

3.3 Quantification of DNA

After extracting DNA from all 42 mango leaf samples using the modified CTAB procedure described above, the DNA's amount and quality were evaluated.

3.3.1 Quantification of DNA using Spectrophotometric method

The source DNA quantified using UV/Vis Nano Spectrophotometer (NABI, Microdigital), purity of DNA checked by measuring the ratio of OD at 260/280ηm. The wavelength ratio of 1.8 indicates pure DNA, higher than 2.0 indicates impurities of RNA and less than 1.8 indicates proteins contamination in the sample. The absorbance at 260 nm allowed the calculation of DNA concentration in the sample.

$$\textbf{DNA (µg/µl)} = \frac{\text{OD at 260 nm x dilution factor x 50}}{1000}$$

3.4 Agarose Gel Electrophoresis (Labindia Instrument Pvt. Ltd)

The Assessment of DNA quality checked using agarose gel electrophoresis (0.8%).

Steps:

Using agarose gel electrophoresis, the DNA quality was evaluated (0.8%).

1. Prepare a 300 ml (0.8% agarose gel) in 1X TAE buffer.
2. On cooling, 4 µL of ethidium bromide were added to the gel.
3. The comb was positioned in the gel-casting tray, which was then filled with gel and let to cool. It takes the gel around 30 min to solidify.
4. The gel wells developed when the comb was gradually withdrawn from the gel-casting tray.
5. A combination of 5 µL of DNA and an equivalent amount of bromophenol blue was loaded into in the wells.
6. To quantify the DNA, a 3µL 1 kb ladder was added to the first and second lanes, respectively.
7. Gel electrophoresis was then performed at 100 volts for 1 h.
8. The gel was then observed using a gel documentation system (Labindia Instrument Pvt. Ltd.).

A.Put the correct amount of agarose into an Erlenmeyer flask by weighing it out. In order to create agarose gels, a w/v percentage solution is used.

B. Agarose concentration in a gel will be influenced by the size of the DNA fragments that will be used. Majority of gels falling between 0.5% and 2% are separated.

C. The buffer's volume shouldn't be more than a third of the flask's capacity.

D. Fill the flask holding the agarose with running buffer. Stir to combine. The most typical TAE (40 mM tris-acetate) 1 mM E. EDTA, TBE (45 mM Tris-borate, 1 mM EDTA) were used as running buffers.

E.Melt the buffer/agarose mixture. Heating in a microwave is the most typical method, however Bunsen flames can also be used. Remove the flask and stir the contents to thoroughly combine it at intervals of 30sec.

F. Continue until all of the agarose has dissolved.

G.Add 0.5 µg/mL of ethidium bromide (EtBr) to the mixture. Alternately, the gel may also be dyed with running buffer following electrophoresis.

H.destaining in a running buffer for an equivalent amount of time after containing 0.5µg/mL EtBr for 15–30 min.

3.4.1 Dilution of DNA sample

The genomic DNA diluted with a suitable quantity of sterile distilled water to yield a working concentration of 20 ηg/µl and kept at -20˚C until further use.

a. A loading the DNA sample separation samples with dye. Gel loading dye is commonly produced at a 6X concentration (0.25% bromphenol blue,

b. 0.25% xylene cyanol, 30% glycerol).

3.4.2 Molecular characterization of wild Mangoes using SSR markers

The technique of SSR amplification is a prerequisite to generate the molecular profiles of various alleles of an individual or genotype. Amplification is the multifold duplication and accumulation of a targeted region which is achieved by a polymerase chain reaction. It needs ingredients such as buffer, $MgCl_2$, dNTPs, primers, and DNA polymerase enzyme. The utilization of these essential PCR components in optimal concentrations determines the success of amplification. Thus SSR primers, helps in enhancing the amplification and thereby generating the genotype profile. With the advent of technology, fluorophore-labeled primers along with automated capillary electrophoresis system have enhanced the efficiency of detection.

3.4.3 Nuclear SSR analysis

Initially 15 primers are selected by considering their location on chromosome and basis of standardization of annealing temperature.

3.5 M13 Tailing

M13 primers were designed by joining a 19bp sequence, CACGACGTTGTAAAACGAC to 5' end of forward primer end and GTTTCTT, a 9 bp sequence to the 5' of reverse primer (Schuelke 2000; Boutin-Ganache *et al*., 2001).

The designed primers were synthesized from M/S Eurofins facility, Bangaluru.

Table 3: Mango SSR primer details

Sl. No.	**Primer ID**	**Forward primer**	**Reverse primer**	**Annealing temperature (˚C)**	**Reference**
Sl.No	**Primer ID**	**Forward (5'-3')**	**Reverse (5'-3')**	**T_a (ºC)**	Ravishankar *et al*. (2015)
1	11588	GTAAACGACGGCCAGTGAGAAATGGTTCCAGCAA	GTTCTTCACGCGAAGTAAACCAAA	56	
2	18463	GTAAAACGACGGCCAGTGGAGACCTCTTGCATCCAT	GTTTCTTCCTTTTTATTGCCGAGTCCA	55	
3	8095	GTAAAACGACGGCCAGTTGAATGGGTAATGGTGAATCG	GTTTCTTTCAACTCAATCTGATCGCTTACA	59	
4	333	GTAAAACGACGGCCAGTAAACCATTGTGGATGTGGGT	GTTTCTTTCCCAGTCTGGAAAAAGAAAAA	56	
5	MiIIHR99	GTAAAACGACGGCCAGTCTTCATCGAATCCAAGGCAT	GTTTCTTCTTCCATGGCACGAGTAGGT	55	
6	MiMRD85	GTAACGACGGCCAGTTTAAAGGGGCTGACAAATGG	GTTTCTTGTGGAGCACTGATTTTTGGG	56	

7	MiKVR 71	GTAAAACGACGGCCAG TAAAAGTGCCACAGAA AACATGTAA	GTTTCTTATGCCTCA ACCTGTTATGCC	60	
8	MiKVR 98	GTAAAACGACGGCCAG TTGATTTCCCCTTAATG TTGATG	GTTTCTTTCATTATC ATGAGTTTCGCCA	55	
9	21478	GTAAAACGACGGCCAG TAAACTTTAATGGATTT TATCGTGTAAT	GTTTCTTAAATACCC CACCCATTTTGC	62	
10	15847	GTAAAACGACGGCCAG TTCTAGAATTTTTGAAA AGATAATGTGA	GTTTCTTTTTCACAT ACAAGTTAGTTGGG G	60	
11	MiIIHR 78	GTAAAACGACGGCCAG TGCCTTGAGGAGGTTTT GACA	GTTTCTTGATGCTTC TTTCCCAACAGC	63	
12	MiMRD 88	GTAAAACGACGGCCAG TAAAATGGACGCCACA AAGTG	GTTTCTTGTTTCGGA TTTCTCATGGGA	54	
13	16400	GTAAAACGACGGCCAG TAAATCCCGTACCTTCA TCCC	GTTTCTTTGCCAGA ACTGCTCTCTTCA	55	
14	MiKVR 80	GTA AAACGACGGCCAGTTA CAGGCTGCCCAGAAAG AT	GTTTCTTGCGCATGC TGGGATTAGTAT	58	
15	MiKVR 81	GTAAAACGACGGCCAG TGTGTTCAGAATACCG GCCAT	GTTTCTTACCCCTGC ATGATTTTGACT	60	

Table 4: Genetic analysis of microsatellite loci using 8 SSRs

Sl. No	**Primer name**	**Primers size**		***T*m(^{o}c)**
		Forward (5'-3')	**Reverse (5'-3')**	

1	11588	GTAAACGACGGCCAGTGAGAAATGGTTCCAGCAA	GTTCTTCACGCGAAGTAAACCAAA	56
2	8095	GTAAAACGACGGCCAGTTGAATGGGTAATGGTGAATCG	GTTTCTTTCAACTCAATCTGATCGCTTACA	59
3	333	GTAAAACGACGGCCAGTAAACCATTGTGGATGTGGGT	GTTTCTTTCCCAGTCTGGAAAAAGAAAAA	56
4	MiIIHR99	GTAAAACGACGGCCAGTCTTCATCGAATCCAAGGCAT	GTTTCTTCTTCCATGGCACGAGTAGGT	55
5	21478	GTAAAACGACGGCCAGTAAACTTTAATGGATTTTATCGTGTAAT	GTTTCTTAAATACCCCACCCATTTTGC	62
6	16400	GTAAAACGACGGCCAGTAAATCCCGTACCTTCATCCC	GTTTCTTTGCCAGAACTGCTCTCTTCA	55
7	MiKVR80	GTAAAACGACGGCCAGTTACAGGCTGCCCAGAAAGAT	GTTTCTTGCGCATGCTGGGATTAGTAT	58
8	MiIIHR17	GCTTGCTTCCAACTGAGACC	GCAAAATGCTCGGAGAAGAC	55

3.5.1 Amplification of genomic DNA of Mango leaves sample using SSR marker

In the PCR tubes, the following quantities of reagents were added to get 20µl of reaction mixture (Table 5).

The reaction mixture was gently vortexed and briefly centrifuged to collect all drops from walls of tube.

The reaction mixtures were placed in a thermocycler (Eppendorf / Bio-ER- Genepro) and PCR was carried out after programming it (Table 6).

Table 5: PCR components utilized in SSR marker analysis

Sl. No	Components	Volume for one reaction (µl)
1	DNA (50ηg/µL)	4
2	10X PCR buffer with $MgCl_2$	2.5
3	dNTP's (1mM)	2
4	Forward primer(5 ρmole)	1
	Reverse primer(5 ρmole)	1
5	Probe (5 ρmole)	0.5
6	*Taq* DNA polymerase(5U/µL)	0.3

7	Nuclease free water	7.2
8	$Mgcl_2$	1.5
9	**Total**	20

Table 6: PCR programme for SSR amplification.

Sl. No	Steps	Temperature (˚C)	Time	No. of cycles
1	Intial denaturation	94	3 min	1
2	Denaturation	94	30 sec	35
3	Annealing	65 or 55	30 sec	
4	Extension	72	30 sec	
5	Final extension	72	5 min	1
6	Hold	4		-

3.5.2 Resolution of amplified product of SSR marker

The PCR amplified product resolved using 1.5% agarose gel having EtBr, at 100v for 1.5 h. Further, image was captured by Gel Documentation System.

3.5.3 Multiplexing

Multiplexing carried out by mixing fourprimers, which are mixed with different probes. Which helps simultaneous detection of amplified product of all these primer at a time. Primers are having M13 tailing sequence in their 5' end, which will bind to dye labeled primers. FAM, VIC, NED and PET are dye bounded to sequence which will bind to M13 sequence of the primer. Dye labeled primers are mixed during PCR in 1;1 ratio with Forward primer. Dye labeled primer which go and bind to the M13 sequence and it give fluorescence (FAM- Blue, VIC-Green, NED-Yellow and PET-Red color). While keeping PCR for one primer one probe is fixed. Multiplexing is a mixing 4 different primers which are having different probes in a single tube.

3.5.3.1 Fragment size analysis (ABI 3730 Automated DNA sequencer, Applied Biosystems)

Fragment size analysis is a crucial technique employed in molecular biology and genetics to determine the size distribution of DNA fragments generated through various experimental processes such as PCR, sequencing, or restriction enzyme digestion. The fragment size analysis was conducted using an ABI 3730 Automated DNA sequencer (Applied Biosystems, California, USA), a widely used platform known for its accuracy and reliability in fragment sizing applications.

The raw data obtained from the ABI 3730 sequencer were processed and analyzed using Peak Scanner software (Applied Biosystems, California, USA), as described by Simko (2009). This software facilitates the extraction of fragment size information from the electropherograms generated by the sequencer, allowing for precise estimation of fragment sizes in base pairs.

To ensure accurate size determination, a size standard (GS500LIZ3730) was included in the analysis. This size standard consists of a mixture of DNA fragments of known sizes, which serve as reference markers for calibrating the electrophoretic mobility of the sample fragments. By comparing the migration patterns of sample fragments with those of the size standard, the Peak Scanner software can accurately assign sizes to the observed peaks in the electropherogram.

The analysis method employed in this study utilized the default parameters provided by the Peak Scanner software, specifically the size default NPP (Non-Proportional Profile) method. This method is designed to handle the analysis of DNA fragments with non-linear mobility profiles, ensuring robust and accurate size estimation even in complex fragment mixtures.

The fragment size analysis using the ABI 3730 sequencer and Peak Scanner software provides a reliable means of characterizing the size distribution of DNA fragments with high precision and reproducibility. This technique is essential for a wide range of molecular biology applications, including genotyping, DNA sequencing, and fragment length polymorphism analysis, and serves as a valuable tool for elucidating genetic diversity and variation in biological samples.

3.6 Statistical analysis

3.6.1 CERVUS 3.0 (Kalinowski *et al*. 2007)

Observed heterozygosity (Ho), the expected heterozygosity (He), and the polymorphic information content (PIC) were calculated using the fragment size data generated for each SSR locus with the help of Cervus 3.0 software (Kalinowski *et al*. 2007). Cervus 3.0.7 is a software program developed by Tristan Marshall, with copyrights spanning from 1998 to 2014. This software is likely designed for a specific purpose, possibly related to data management,.

Fragment size data for SSR (Simple Sequence Repeat) loci are essential for various genetic analyses, particularly in population genetics and parentage studies. The software

CERVUS 3.0, developed by Kalinowski et al. In 2007, uses such data to perform statistical analyzes for estimating genetic diversity, relatedness, and assigning parentage in populations.

Fragment size data for SSR loci serve as fundamental inputs for genetic analysis software like CERVUS 3.0. These data are crucial for accurately estimating various population genetic parameters, such as allele frequencies, genetic diversity, and relatedness among individuals. This descriptive overview highlights the significance of fragment size data in genetic studies and their application in the CERVUS 3.0 software.

SSR loci, also known as microsatellites, are regions within the genome characterized by short, repeated DNA sequences. These loci exhibit high levels of polymorphism, making them valuable markers for studying genetic variation within and among populations. CERVUS 3.0 is a widely used software tool that employs fragment size data from SSR loci to conduct genetic analyses, including parentage assignment, individual identification, and population genetic analysis.

Fragment size data for SSR loci are typically obtained through polymerase chain reaction (PCR) amplification of target DNA regions followed by fragment analysis using capillary electrophoresis. The resulting data consists of allele sizes corresponding to the length of PCR-amplified fragments, which are used as input for CERVUS 3.0 analysis.

In CERVUS 3.0 analysis, fragment size data are used to estimate allele frequencies within populations, calculate genetic diversity indices such as expected heterozygosity and allelic richness, and determine pairwise relatedness among individuals. Additionally, CERVUS 3.0 employs likelihood-based methods to assign parentage and identify potential candidates for parent-offspring relationships based on matching fragment size data.

Fragment size data for SSR loci are invaluable for understanding genetic diversity, population structure, and individual relationships in natural populations. The integration of such data into software platforms like CERVUS 3.0 enhances the efficiency and accuracy of genetic analysis, facilitating a wide range of applications in ecology, conservation biology, and forensics. By providing insights into population genetic parameters and individual relationships, these data contribute to our understanding of evolutionary processes and aid in practical applications such as wildlife management and forensic investigations.

3.6.3 AMOVA

AMOVA (Analysis of Molecular Variance) is a statistical method that is commonly used in population genetics to partition genetic variation within and among populations. It is a useful tool for investigating the genetic structure and diversity of populations and can be applied to a wide range of genetic data, including microsatellites, single nucleotide polymorphisms

(SNPs), and mitochondrial DNA sequences. AMOVA is based on the analysis of molecular variance, which quantifies the genetic variation within and among populations by calculating the variance components. The AMOVA method uses a hierarchical approach to partition genetic variation into different levels of organization, such as among populations, within populations, and among individuals. The AMOVA approach can also be used to test the significance of genetic differentiation among populations and to estimate gene flow rates.

3.6.3.1 AMOVA analysis typically involves four steps

(1) data input and formatting

(2) calculation of genetic distances or divergences

(3)analysis of molecular variance and

(4) statistical testing of the significance of the variance components.

The output of AMOVA includes estimates of variance components, F-statistics (FST, FIS, and FIT), and p-values.AMOVA has several advantages over other methods of analyzing genetic variation, including its ability to incorporate hierarchical population structure, its robustness to missing data, and its ability to handle large datasets. However, AMOVA also has some limitations, including its sensitivity to the choice of genetic marker and the assumption of a certain level of equilibrium between genetic drift and gene flow.

AMOVA is a powerful tool for investigating the genetic structure and diversity of populations and can provide valuable insights into the evolutionary history and ecology of species. When used appropriately, AMOVA can be a valuable addition to the arsenal of statistical methods available to population geneticists and evolutionary biologists using GenAlEx 6.503 software.

3.6.4 Principal Component Analysis

Principal Component Analysis (PCA) is a powerful statistical technique employed in molecular studies to simplify complex datasets while preserving essential information using XLStat 2022. By transforming original variables into a smaller set of uncorrelated principal components, PCA reduces dimensionality, highlighting key patterns and relationships within molecular data. This method is particularly valuable in genomics, proteomics, and metabolomics, where high-dimensional data is common. PCA aids in visualizing variations, identifying significant biomarkers, and uncovering underlying biological processes. Its ability to enhance data interpretability and reveal hidden structures makes PCA a fundamental tool in advancing molecular research and understanding intricate biological systems.

Principal Component Analysis (PCA) involves several key steps to transform and analyze high-dimensional data. Here are the detailed steps involved in PCA:

Standardize the Data:Since PCA is affected by the scale of the variables, standardizing the data (i.e., centering the data by subtracting the mean and scaling to unit variance) ensures that each variable contributes equally to the analysis.

Compute the Covariance Matrix: Calculate the covariance matrix to understand how variables in the dataset relate to one another. The covariance matrix captures the pairwise covariances between variables.

Calculate the Eigenvalues and Eigenvectors: Compute the eigenvalues and eigenvectors of the covariance matrix. Eigenvectors (principal components) determine the directions of the new feature space, while eigenvalues measure the magnitude of the variance along each eigenvector.

Sort Eigenvalues and Eigenvectors: Sort the eigenvalues in descending order and arrange the corresponding eigenvectors accordingly. The eigenvectors with the highest eigenvalues represent the directions of maximum variance.

Select Main Components: Choose the top k eigenvectors (principal components) that correspond to the k largest eigenvalues. The number of principal components selected depends on the amount of variance one aims to retain in the data.

Transform the Data: Project the original standardized data onto the selected principal components to form a new dataset. This transformation reduces the dimensionality of the data while retaining most of the original variance.

Analyze the Results: Interpret the main components and their contributions to understanding the structure of the data. Visualize the results through plots such as scatter plots or scree plots to identify patterns, clusters, and outliers.

3.6.5 Principal Coordinate Analysis

Principal Coordinate Analysis (PCoA) is a valuable method used in molecular diversity studies to visualize and interpret the differences among samples based on their molecular characteristics using GenALEX 60503. Here's an overview of how PCoA is applied in this context:

Steps are as follows

Data Collection and Preprocessing: Obtain molecular data, such as genetic sequences, protein expressions, or metabolite profiles, from different samples. Preprocess the data to normalize

or standardize it if necessary, ensuring that variations are due to biological differences rather than technical biases.

Distance Matrix Calculation:Compute a pairwise distance matrix that quantifies the dissimilarity between each pair of samples. Common distance measures include:

Bray-Curtis distance for ecological data.

Jaccard index for presence/absence data.

Euclidean distance for continuous data.

Double-Centering the Distance Matrix: Transform the distance matrix into a centered matrix to ensure that the data is ready for eigenvalue decomposition. This step involves subtracting the row means, column means, and adding the overall meaning of the matrix.

Eigenvalue and Eigenvector Computation: Perform eigenvalue decomposition on the centered distance matrix to obtain eigenvalues and eigenvectors. The eigenvalues represent the amount of variation explained by each principal coordinate, while the eigenvectors define the directions of the principal coordinates.

Selection of Principal Coordinates: Select the principal coordinates that correspond to the largest eigenvalues. These principal coordinates capture the most significant dimensions of variation in the data.

Data Projection: Project the original molecular data onto the selected principal coordinates to create a new dataset in a reduced-dimensional space. Each point in this space represents a sample.

Visualization and Interpretation: Create scatter plots or 3D plots of the principal coordinates to visualize the relationships between samples. This helps identify patterns, clusters, and outliers. Analyze the plot to interpret the molecular diversity, looking for groupings that correspond to different biological conditions, treatments, or populations.

3.6.2 Cluster analysis (Perrier and Jacquemoud 2006)

SSR genotypic data used for cluster analysis using neighbor-joining (NJ) tree based on simple matching (SM) dissimilarity matrix using DARwin software version 6.0.10 (Perrierand Jacquemoud, 2006).

Cluster analysis based on SSR (Simple Sequence Repeat) genotypic data explains about the genetic relationships among individuals or populations. The Neighbor-Joining (NJ) method, coupled with a simple matching (SM) dissimilarity matrix, offers a robust approach for visualizing genetic distances and clustering patterns. This note discusses the application of DARwin software version 6.0.10, developed by Perrier and Jacquemoud (2006), for constructing NJ trees from SSR genotypic data and interpreting the resulting clusters.

SSR markers are widely used in population genetics and plant breeding due to their high variability and co-dominant inheritance. Cluster analysis based on SSR genotypic data allows researchers to explore genetic relationships, population structure, and diversity patterns. The Neighbor-Joining algorithm, a popular phylogenetic method, constructs hierarchical trees to represent genetic distances among individuals or populations. DARwin (Dissimilarity Analysis and Representation for Windows) software provides a user-friendly platform for conducting cluster analysis and visualizing genetic diversity using various molecular marker datasets, including SSRs.

The SSR genotypic data are typically obtained through PCR amplification of SSR loci followed by fragment analysis. The resulting genotype data matrix represents the presence or absence of alleles at each SSR locus for each individual or population. DARwin software version 6.0.10 allows users to input SSR genotype data and compute a simple matching dissimilarity matrix, which quantifies the genetic distances between pairs of individuals or populations based on allele sharing. Using the simple matching dissimilarity matrix, DARwin constructs a Neighbor-Joining tree that visually represents the genetic relationships among individuals or populations. Clusters of closely related individuals or populations appear as branches with shorter branch lengths, while more distantly related groups are represented by longer branches. The resulting NJ tree provides insights into the genetic structure, diversity, and evolutionary relationships within the studied populations.

Cluster analysis with NJ tree construction using SSR genotypic data in DARwin software offers a powerful approach for understanding genetic relationships and population structure. The resulting tree can aid in the identification of genetically distinct populations, the assessment of gene flow among populations, and the selection of individuals for breeding or conservation purposes. Additionally, DARwin's user-friendly interface and visualization tools facilitate the interpretation and communication of complex genetic data to diverse audiences.

Utilizing SSR genotypic data for cluster analysis with NJ tree construction in DARwin software enables researchers to explore genetic relationships and population structure effectively. By leveraging the simplicity and robustness of the NJ method and the user-friendly features of DARwin, this approach contributes to our understanding of genetic diversity, evolutionary processes, and the management of genetic resources in various organisms.

4. RESULTS

4.1 Eco-Geographic Survey

An Eco-Geographic survey was conducted during April, May and June 2019 following simple random strategy, the fresh young leaves and ripen fruits of wild *Mangifera indica* L.,were collected from different locations of Dakshina kannada and Udupi districts of Karnataka state as represented as in table 2,3,4,5 with Global Positioning System;

TABLE 2 : Wild mango sample survey locations from Bantwal Taluk

SI.NO	Varieties	Locations	Global Positioning
B1	**Wild mango 1**	Koragatte	12º57’51.99″ 75º5’52.623″
B2		Vagga	12º91’40.88″ 75º12’69.20″
B3		Dhande	12º94’24.89″ 75º08,80.02″
B4		Kodiyadka	12º94’49.29″ 75º08’69.45″
B5		Siddakatte	13º0’2.5956″ 75º3’3.7836″
B6		Karimale	12º95’.7888″ 75º0’7.0839″
B7		Ananthady	12º48’36.84″ 75º06’22.11″
B8		Nelyakumar	12º58’25.04″ 75º04’14.36″
B9		Korey	12º94’37.37″ 75º08’69.56″
B10		Kuthkoli	12º9’80.030″ 75º08’69.56″
B11		Padil	12º56’55.19″ 75º05’22.07″
B12		Iravaje	12º95’75.61″ 75º11’09.35″

B13		Pilimogaru	12º94’77.6″ 75º10’77.8″

TABLE 3 : Wild mango sample survey locations from Hebri Taluk

SI.NO	Varieties	Locations	Global Positoning
H1		Kelakila	13º24’50.46″ 75º01’2.79″
H2		Hebri	13º47’42.64″ 75º03’95.32″
H3		Adishakthi mudradi	13º25’58.01″ 75º0’30.98″
H4		Mudradi	13º26’8.80″ 75º01’56.22″
H5		Bailumane-1	13º45’55.83″ 75º05’37.87″
H6		Sitanadi	13º47’56.38″ 75º02’72.83″
H7		Muniyal	13º21’20.82″ 75º01’12.77″
H8		Bailumane 2	13º45’06.67″ 75º06’76.86″
H9		Sitanadi 1	13º47’62.17″ 75º02’92.08″
H10		Athrady	13º26’03.0″ 74º56’44.0″
H11		Parkala	13º45’55.83″ 75º05’37.87″

TABLE 4 : Wild mango sample survey locations from Karkala Taluk

SI.NO	Varieties	Locations	Global Positoning
K1		Yennehole	13º17’38.54″ 74º59’6.54″
K2		Jodurasthe	13º12’2.17″ 75º11’52.95″
K3		Jodukatte	12º58’14.15″ 75º03’57.49″
K4		Anekere	13º11’50.53″ 75º1’12.48″
K5		Karkala bypass	13º11’24.14″ 74º59’43.90″
K6		Pulkeri	13º11’39″ 74º59’43″
K7		Miyar kuntibail	13º19’79″ 75º03’58″
K8		Borugudde	13º1’9.79″ 75º0’3.58″
K9		Bannadka	13º5’50.67″ 74º59’56.80″
K10		Beluvayi	13º7’2.06″ 74º59’42.78″
K11		Kesargaddhe	13º11’22.64″ 74º99’75.97″

TABLE 5 : Wild mango sample survey locations from Moodbidri Taluk

SI.NO	Varieties	Locations	Global Positoning
M1		Sampige	74º57’28.04″ 13º03’37.53″
M2		Masthikatte	13º03’42.36″ 74º57’45.96″
M3		Thacode 1	13º03’55.75″ 75º03’13.60″
M4		Thacode 2	13º21’47.84″ 75º20’19.86″
M5		Meginamane	13º4’7.67″ 74º59’36.95″
M6		Alangar	13º08’33.98″ 74º99’34.27″
M7		Birav	13º08’33.09″ 74º97’18.36″
M8		Paper mill	13º1’58.43″ 75º2’0.25″
M9		Ontikatte	13º3’48.24″ 75º0’12.51″
M10		Laadi	13º06’34.21″ 74º99’84.97″
M11		Kodiyadka	13º08’24.65″ 74º96’88.33″

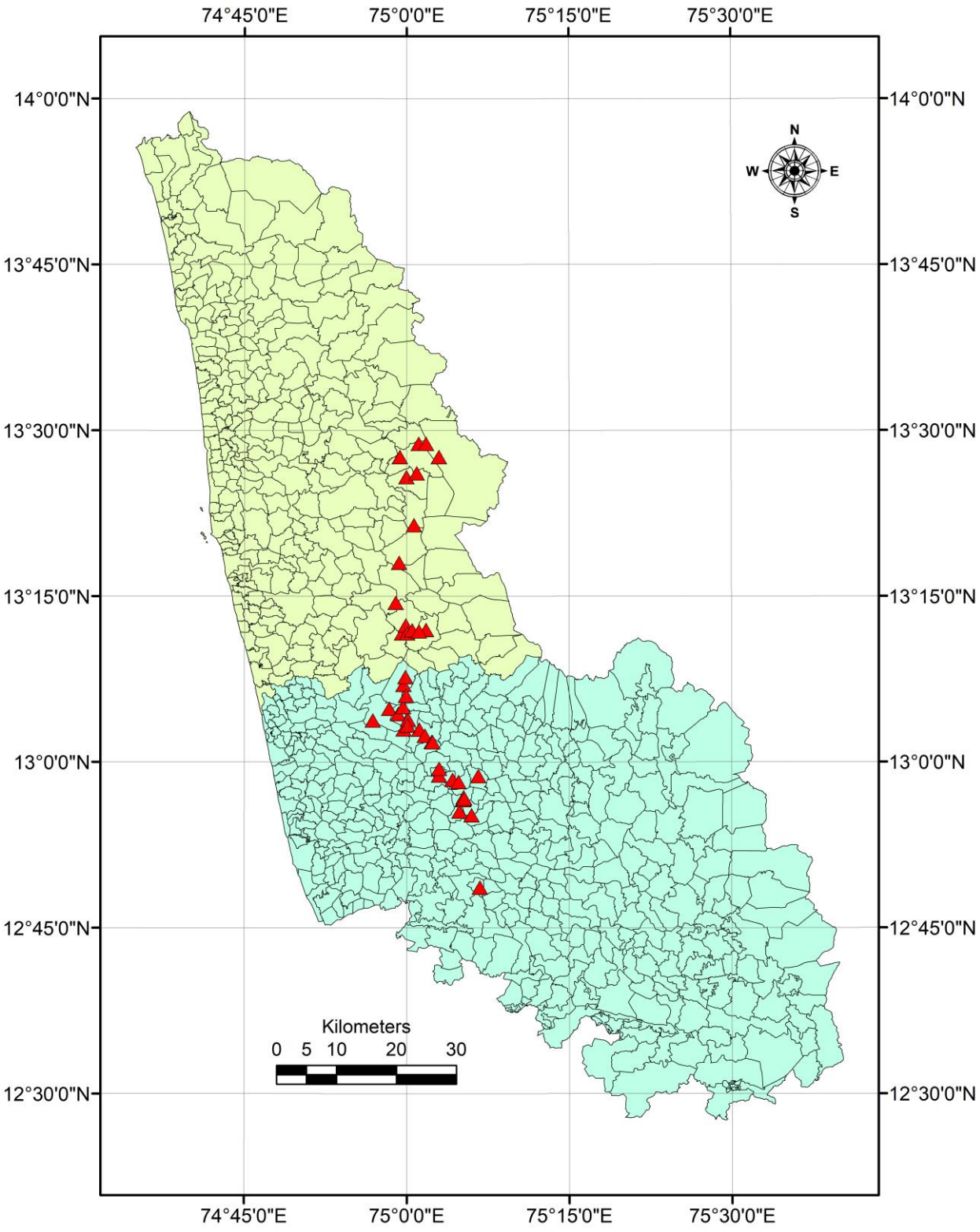

Figure 1: Sampling sites of Dakshina Kannada and Udupi districts of Karnataka

4.2 Morphological Characterization of Mango Germplasm Varieties

The results obtained from the present investigation are presented and studied on different plant varieties of coastal districts in Karnataka and it has provided a comprehensive list of qualitative characteristics of various mango varieties. The considerable variation in mango fruits and trees represented in plate 1 and plate 2. The variation in skin color, pulp color, fruit apex, fruit shape, fruit beak type, and skin thickness was also observed. These observations have significant implications for the conservation and management of plant genetic resources, particularly in the face of changing environmental conditions and the need for sustainable agriculture.

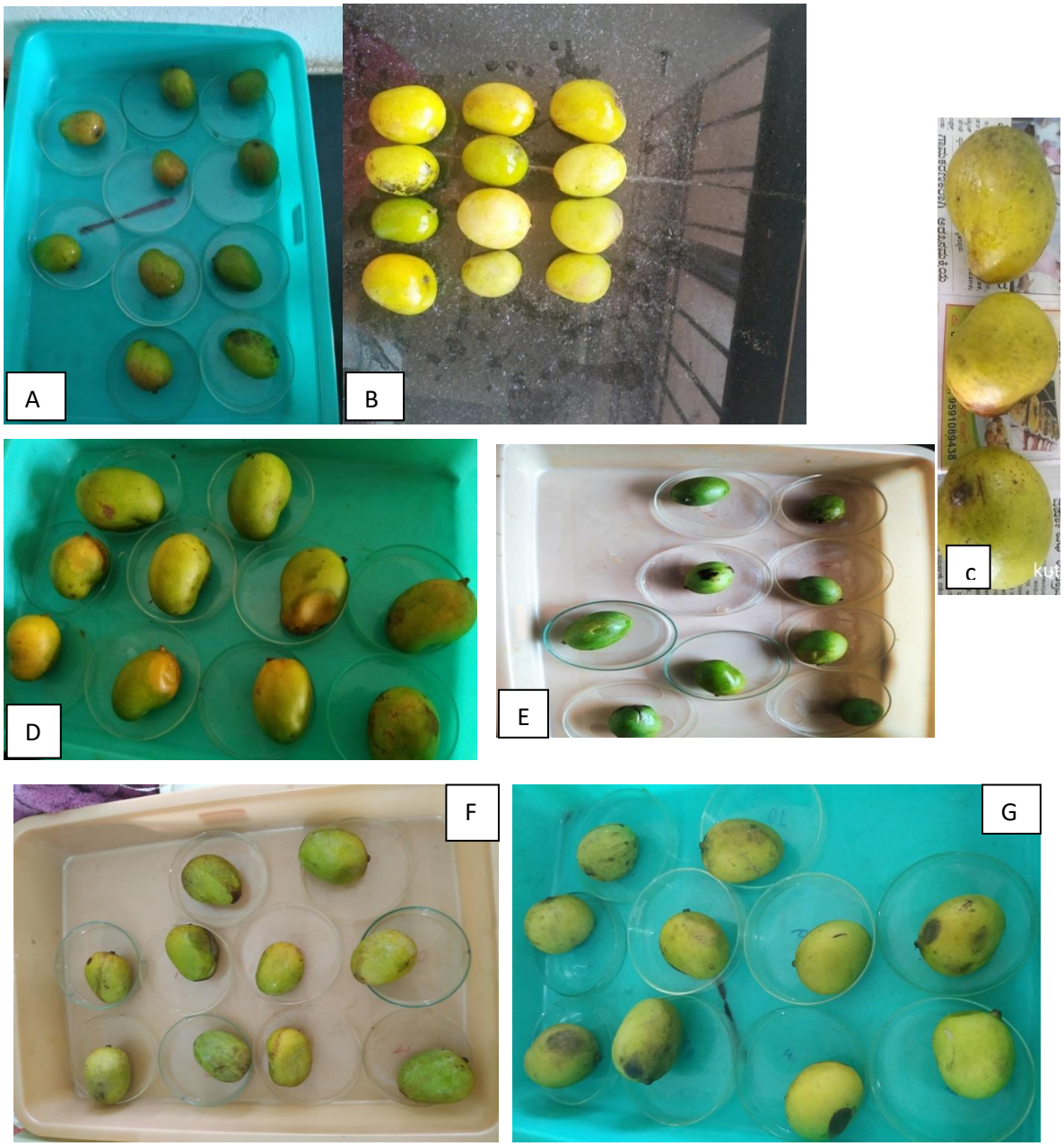

Plate 1: Wild mango fruits of wild mango varieties of Dakshina Kannada and Udupi

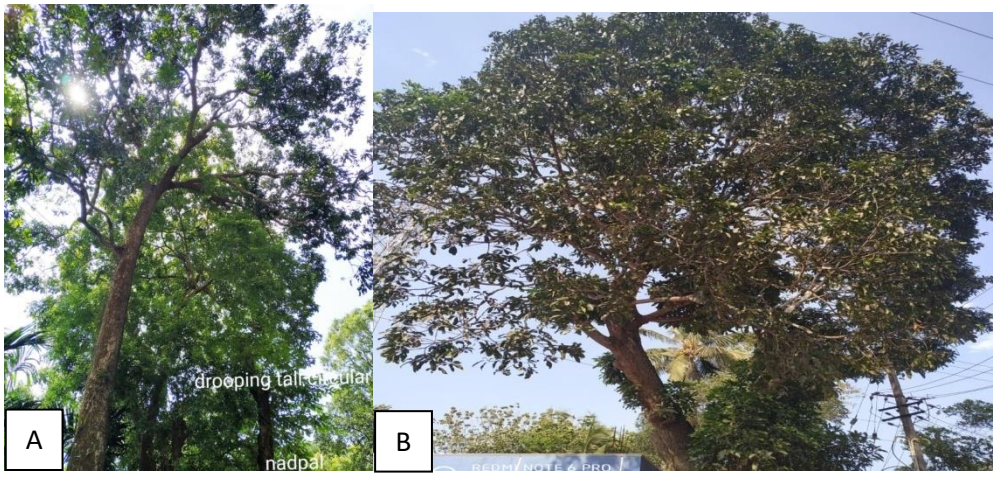

Plate 2: Wild mango tress and wild mango varieties of Dakshina Kannada and Udupi districts

The qualitative characteristics and variations in fruit shape, color, and pulp color, along with other plant traits such as tree height, crown shape, leaf apex shape, leaf base shape and leaf fragrance of wild mangoes of coastal districts in Karnataka was observed. There is a considerable variation in skin color with most varieties exhibiting a yellowish to greenish appearance, yellowish-green to yellow, green, and even red. It was revealed that the most common skin color of the mango varieties was green with 16 varieties showing that color. The other prevalent skin colors were yellowish-green with 10 varieties and greenish-yellow with 8 varieties. Other skin colors observed in the study were pale yellowish, greenish pale yellow, and yellowish-orange, with 1, 6, and 3 varieties are reepresented in Figure 5. Similarly, the pulp color represented in figure 6 and it is ranged from yellowish to orange, with fewer variations observed in fruit characteristics such as fruit apex which is usually roundish in shape. A range of pulp colors among different mango varieties with orange being the most common color followed by pale yellow (8 varieties). Some varieties also exhibited a whitish-yellow (2 varieties) pulp color while others showed an orange (19 varieties), yellow (8 varieties), yellow orange (6 varieties), pale orange (1 variety), orange yellow (1 variety). The fruit shape of the varieties exhibited in figure 8 and range the different shapes including round, elliptic, ovoid, oblong, and obtuse. The most common fruit shape observed in the current study was round with 32 varieties exhibiting this shape.

The qualitative characteristics of mango varieties of coastal districts in Karnataka into their qualitative characteristics. The fruit characteristics such as fruit apex which is usually roundish in shape. Thetree height represented in figure 7 and the majority of varieties were medium in height with 20 varieties falling in this category. However, there were also a significant number of varieties that were tall (16 varieties) and very tall (9 varieties). The

crown shape of the different plant varieties was investigated and a variety of shapes, including semicircular (13 varieties), spherical (7 varieties), circular (7 varieties), broadly pyramidal (9 varieties), oblong (8 varieties), and erect (1 variety) were found. The leaf apex shape and leaf base shape were also analyzed in the figure 2 and figure3 with the majority of varieties exhibiting acute leaf base shape (37 varieties), obtuse (7 varieties) and a while one variety had round leaf base. Leaf apex shape, acuminate (31 varieties), acute (10 varieties), obtuse (3 varieties),The plant species exhibit a wide range of leaf shapes which may play a role in their adaptation to specific environmental conditions. The mild fragrance of the plant leaves was found in 18 varieties while 26 varieties had no fragrance at all shown in Figure 4. In these exhibits the plant species show a wide range of variations in fruit shape, color, and pulp color as well as other plant traits such as tree height, crown shape, leaf apex shape, leaf base shape, and leaf fragrance. These observations highlight the importance of preserving the natural genetic diversity of plant species which may have significant implications for their adaptation to changing environmental conditions.

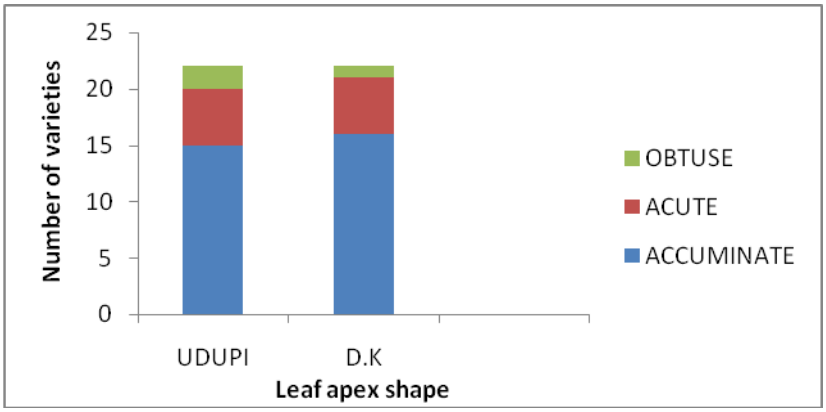

Figure 2: The leaf apex shapes of wild mango varieties of Dakshina Kannada and Udupi. (IPGRI 2006)

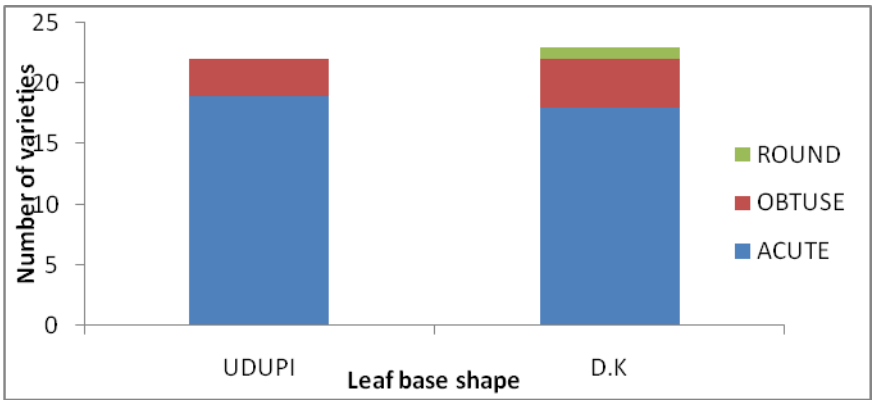

Figure 3: The leaf base shape of wild mango varieties of Udupi and Dakshina Kannada (IPGRI, 2006)

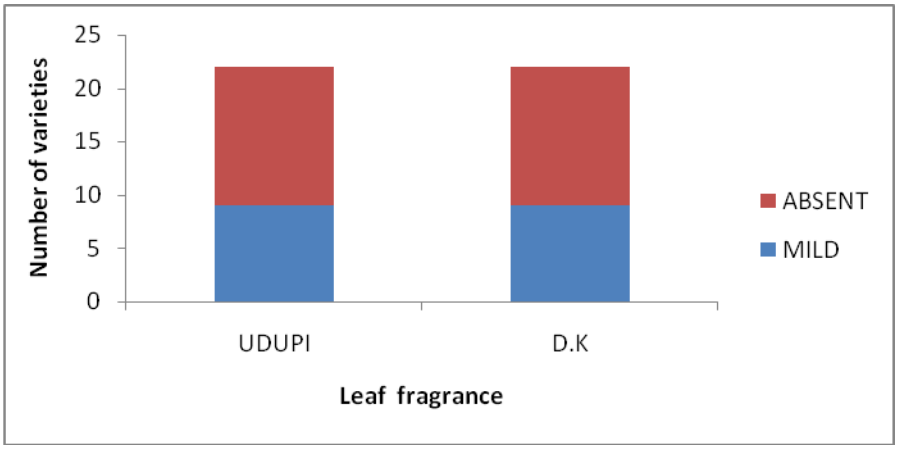

Figure 4: The leaf fragrance of wild mango varieties of Udupi and Dakshina Kannada (IPGRI 2006)

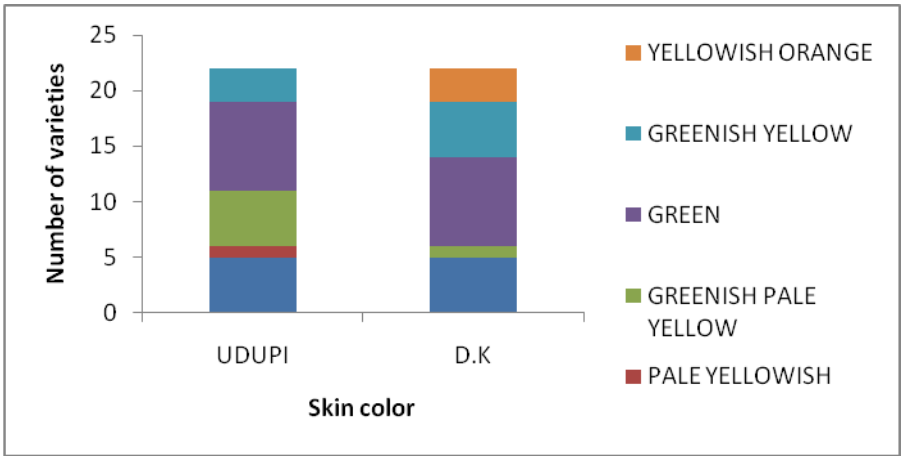

Figure 5: The skin color of wild mango varieties of Udupi and Dakshina Kannada(IPGRI 2006)

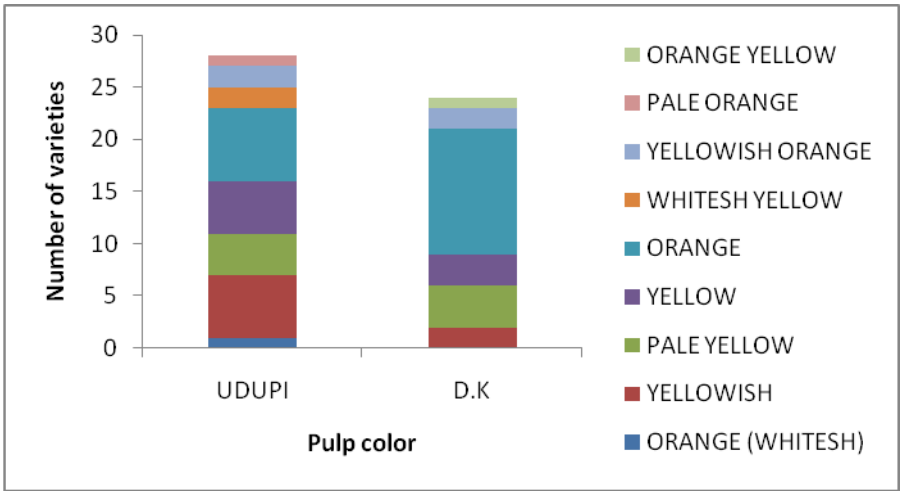

Figure 6: The pulp color of wild mango varieties of Udupi and Dakshina Kannada

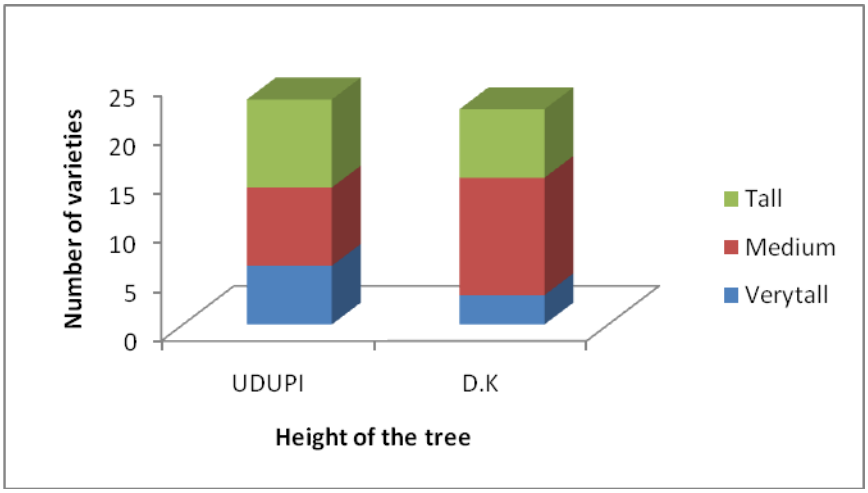

Figure 7: The height of the tree of wild mango varieties of Udupi and Dakshina Kannada (IPGRI 2006)

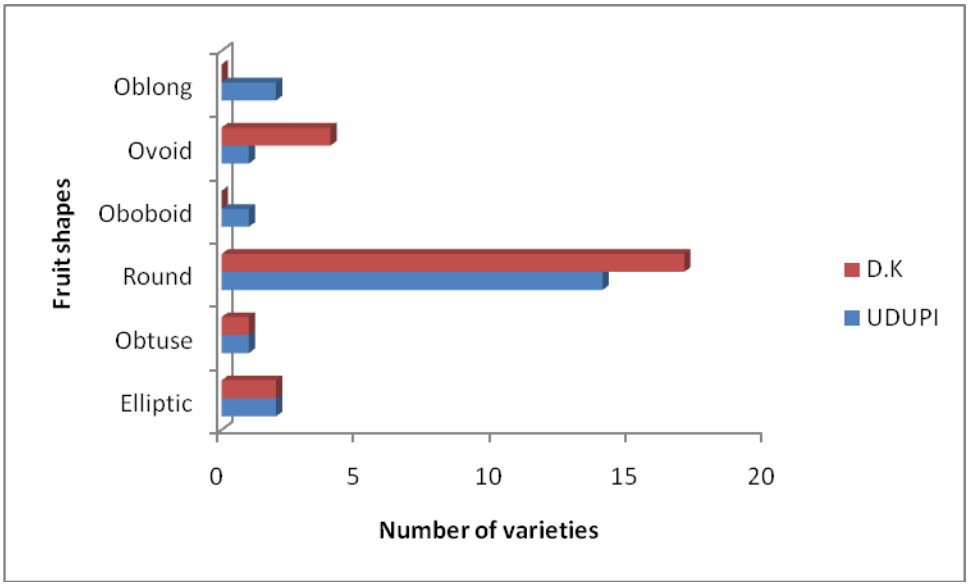

Figure 8: The fruit shapes of wild mango varieties of Udupi and Dakshina Kannada (IPGRI 2006)

The variations in fruit apex and shape among the different mango varieties was given in table 6 and 7. The fruit apex of 45 genotypes was found to be roundish. The study also revealed that the fruit beak type of most varieties was perceptible with 43 varieties are showing this characteristic. Some varieties showed a pointed beak type while others had a round beak type. In terms of skin thickness the study found that the most common thickness of mango skin was 0.2 with 25 varieties exhibiting this thickness. The other thicknesses observed were 0.1 and 0.3 with 18 and 2 varieties respectively

Table 6: List of qualitative characters of different plant varieties of Udupi (IPGRI 2006)

	Fruit Apex	**Crown Shape**	**Fruit Beak Type**
Kelakila	Roundish	Erect	Perceptible
Hebri	Roundish	Oblong	Perceptible
Adishakthi mudradi	Roundish	Semi circular	Perceptible
Mudradi	Roundish	Circular	Perceptible
Bailumane-1	Roundish	Circular	Perceptible
Sitanadi	Roundish	Semi circular	Perceptible
Muniyal	Roundish	Semi circular	Perceptible
Bailumane 2	Roundish	Semi circular	Perceptible
Sitanadi	Roundish	Circular	Perceptible
Athrady	Roundish	Semi circular	Perceptible
Parkala	Roundish	Broadly pyramidal	Perceptible
Yennehole	Roundish	Semi circular	Perceptible
Jodurasthe	Roundish	Spherical	Perceptible
Jodukatte	Roundish	Semi circular	Perceptible
Anekere	Roundish	Circular	Perceptible
Karkala bypass	Roundish	Broadly pyramidal	Perceptible
Pulkeri	Roundish	Semi circular	Perceptible
Miyar kuntibail	Roundish	Spherical	Round
Borugudde	Roundish	Circular	Perceptible
Bannadka	Roundish	Semi circular	Perceptible
Beluvayi	Roundish	Spherical	Perceptible
Kesargaddhe	Roundish	Circular	Perceptible

Table 7: List of qualitative characters of different plant varieties of Dakshina Kannada (IPGRI 2006)

Sampige	Roundish	Broadly pyramidal	Perceptible
Puthige	Roundish	Spherical	Perceptible
Thacode 1	Roundish	Oblong	Perceptible
Thacode 2	Roundish	Spherical	Perceptible
Meginamane	Roundish	Broadly pyramidal	Perceptible

Alangar	Roundish	Oblong	Perceptible
Birav	Roundish	Spherical	Perceptible
Papermill	Roundish	Broadly pyramidal	Perceptible
Ontikatte	Roundish	Broadly pyramidal	Perceptible
Laadi	Roundish	Oblong	Perceptible
Kodiyadka	Roundish	Broadly pyramidal	Perceptible
Koragatte	Roundish	Oblong	Perceptible
Vagga	Roundish	Oblong	Perceptible
Dhande	Roundish	Broadly pyramidal	Pointed
Kodiyadka	Roundish	Semi circular	Perceptible
Siddakatte	Roundish	Oblong	Perceptible
Karimale	Roundish	Broadly pyramidal	Perceptible
Ananthady	Roundish	Spherical	Perceptible
Nelyakumar	Roundish	Circular	Perceptible
Korey	Roundish	Semi circular	Perceptible
Kuthkoli	Roundish	Oblong	Perceptible
Padil	Roundish	Semi circular	Perceptible
Iravaje	Roundish	Semi circular	Perceptible

In the 45 mango varieties morphological traits expressed in qualitative and quantitative characters of leaf and fruit examined with numerical method represented in Table 8 The values of mean and standard deviation of 14 characters viz., leaf blade length, leaf blade width, petiole length, fruit length, fruit diameter, fruit weight, fruit width, pulp, TSS, peel, fruit thickness, stone length, stone thickness, stone weight are given in above Table 8. The observations are in mango genotypes expressed in morphological variations from genotype to genotype (Bhamini *et al*. 2018). The average leaf blade lengths differ across districts, with Moodbidri exhibiting the longest leaf blades (20.97±3.66 cm), followed by Bantwal (18.66±3.83 cm), Karkala (17.64±4.78 cm), and Hebri (15.41± 3.07 cm).Bantwal has the widest leaf blades (5.08±0.85 cm), followed by Moodbidri (4.83±0.52 cm), Karkala (4.59±0.95 cm), and Hebri (4.26±0.88 cm).Petioles are longest in Bantwal (3.26±0.81 cm), followed by Karkala (3.01±0.95 cm), Moodbidri (2.98±0.47 cm), and Hebri (2.28±0.67 cm).Fruit dimensions vary across districts, with Karkala having the largest fruit diameter (15.32±4.76 cm) and Bantwal having the highest fruit weight (72.86±30.96 g). Moodbidri

exhibits the highest pulp content (16.41±10.48%) and the highest TSS (Brix) value (19.05±2.97).Peel percentage is highest in Karkala (23.59±9.08%) while Moodbidri shows the lowest variation in fruit thickness (3.45±0.88 cm). Stone dimensions and weight also vary, with Karkala exhibiting the longest stones (5.14±1.017 cm) and the highest stone weight (32.68±13.35 g).

Table 8: Comparison between wild mango genotypes parameters* of coastal Districts of Karnataka

	Udupi		Dakshina Kannada	
Parameter	Hebri	Karkala	Moodbidri	Bantwal
Leaf blade length (cm)	15.41± 3.07	17.64±4.78	20.97± 3.66	18.66±3.83
Leaf blade width (cm)	4.26± 0.88	4.59±0.95	4.83±0.52	5.08±0.85
Petiole length (cm)	2.28±0.67	3.01±0.95	2.98±0.47	3.26±0.81
Fruit length (cm)	5.36±0.69	5.61±0.97	5.22±0.48	5.31±0.91
Fruit diameter (cm)	12.82±1.67	15.32±4.76	12.58±3.66	14.29±2.11
Fruit weight (g)	56.75±19.55	73.57±27.53	60.69±15.34	72.86±30.96
Fruit width (cm)	3.89±0.57	4.38±0.89	4.59±0.68	4.69±0.71
Pulp (%)	11.08±6.35	14.68±5.86	16.41±10.48	18.46±12.32
TSS (brix)	17.44±3.96	18.01±3.36	19.05±2.97	18.23±3.06
Peel (%)	17.49±6.78	23.59±9.08	19.49±4.88	20.44±7.05
Fruit thickness (cm)	3.84±0.46	4.14±0.57	3.45±0.88	3.55±1.15
Stone length (cm)	4.97±0.66	5.14±1.017	4.71±0.37	4.88±0.72
Stone thickness (cm)	2.03±0.23	2.11±0.26	1.33±0.81	1.73±0.68
Stone weight (g)	25.88±7.22	32.68±13.35	24.20±4.53	29.80±11.85

***Each parameter expressed as Mean±SD; N=5**

The mean and CV of qualitative and quantitative characteristics of D.K and Udupi given table 9. Moodbidri exhibits the highest mean leaf blade length (20.97) with a high C.V. (27.13%), while Hebri has the lowest mean (15.41) with a low C.V. (17.45%). Bantwal has the highest mean leaf blade width (5.08) with a high C.V. (20.73%), whereas Hebri has the lowest mean (4.26) with a relatively lower C.V. (10.81%).Bantwal demonstrates the highest mean petiole length (3.25) with a high C.V. (30.59%), whereas Hebri has the lowest mean (2.28) with a comparatively lower C.V. (15.89%).Karkala

showcases the highest mean fruit length (5.61) with a high C.V. (16.86%), while Moodbidri has the lowest mean (5.22) with a lower C.V. (9.22%). Karkala displays the highest mean fruit diameter (15.32) with a high C.V. (31.05%), whereas Moodbidri has the lowest mean (12.58) with a moderate C.V. (13.04%). Karkala represents the highest mean fruit weight (73.57) with a moderate C.V. (42.49%), while Moodbidri has the lowest mean (56.76) with a relatively lower C.V. (25.23%). Karkala demonstrates the highest mean fruit width (4.69) with a high C.V. (20.53%), while Hebri has the lowest mean (3.89) with a moderate C.V. (14.78%). Bantwal shows the highest mean pulp percentage (18.46) with a high C.V. (66.74%), whereas Hebri has the lowest mean (11.08) with a relatively lower C.V. (39.92%). Moodbidri exhibits the highest mean TSS (19.05) with a high C.V. (22.70%), while Hebri has the lowest mean (17.44) with a moderate C.V. (15.58%). Karkala demonstrates the highest mean peel percentage (23.58) with a high C.V. (38.78%), whereas Hebri has the lowest mean (17.49) with a moderate C.V. (25.05%). Karkala showcases the highest mean fruit thickness (4.12) with a high C.V. (32.45%), while Moodbidri has the lowest mean (3.45) with a moderate C.V. (12.16%). Karkala displays the highest mean stone length (5.14) with a moderate C.V. (19.79%), while Moodbidri has the lowest mean (4.71) with a relatively lower C.V. (7.86%).

Karkala represents the highest mean stone thickness (2.17) with a very high C.V. (61.22%), whereas Moodbidri has the lowest mean (1.32) with a moderate C.V. (11.44%).Karkala showcases the highest mean stone weight (32.68) with a moderate C.V. (40.88%), whereas Moodbidri has the lowest mean (24.20) with a relatively lower C.V. (18.72%).

Table 9: Comparison of Mean and C.V of various Characteristics of mango leaf and fruits of four taluks of Dakshina Kannada and Udupi

		Mean		C.V	
Characteristics		High	Low	High	Low
Leaf blade length (cm)	Measure	20.97	15.41	27.13	17.45
	Area	Moodbidri	Hebri	Karkala	Moodbidri
Leaf Blade width (cm)	Measure	5.08	4.26	20.73	10.81
	Area	Bantwal	Hebri	Hebri	Moodbidri
Petiole length (cm)	Measure	3.25	2.28	30.59	15.89
	Area	Bantwal	Hebri	Karkala	Moodbidri
Fruit length	Measure	5.61	5.22	16.86	9.22

(cm)	Area	Karkala	Moodbidri	Bantwal	Moodbidri
Fruit diameter (cm)	Measure	15.32	12.58	31.05	13.04
	Area	Karkala	Moodbidri	Bantwal	Moodbidri
Fruit weight (g)	Measure	73.57	56.76	42.49	25.23
	Area	Karkala	Moodbidri	Karkala	Hebri
Fruit width (cm)	Measure	4.69	3.89	20.53	14.78
	Area	Karkala	Hebri	Bantwal	Moodbidri
Pulp percentage (%)	Measure	18.46	11.08	66.74	39.92
	Area	Bantwal	Hebri	Bantwal	Karkala
TSS (brix)	Measure	19.05	17.44	22.70	15.58
	Area	Moodbidri	Hebri	Hebri	Moodbidri
Peel percentage (%)	Measure	23.58	17.49	38.78	25.05
	Area	Karkala	Hebri	Hebri	Moodbidri
Fruit thickness (cm)	Measure	4.12	3.45	32.45	12.16
	Area	Karkala	Moodbidri	Bantwal	Hebri
Stone length (cm)	Measure	5.14	4.71	19.79	7.86
	Area	Karkala	Moodbidri	Karkala	Moodbidri
Stone thickness (cm)	Measure	2.17	1.32	61.22	11.44
	Area	Karkala	Moodbidri	Bantwal	Hebri
Stone weight (g)	Measure	32.68	24.20	40.88	18.72
	Area	Karkala	Moodbidri	Karkala	Moodbidri

It was analyzed the quantitative morphological traits of leaf, petiole, fruit, and stones of mango populations from two regions in Karnataka, India - Dakshina Kannada and Udupi in Table 10 It has been calculated the Coefficient of Variation (CV), Mean, and Standard Deviation (SD) for each trait to understand the variability and potential for selective breeding efforts. There is found that the combined CV of Total Soluble Solids (TSS), Fruit thickness, and Stone thickness was higher in Dakshina Kannada than in Udupi. The combined CV of Leaf blade length, Leaf blade width, Petiole length, Fruit length, Fruit diameter, Fruit weight, Fruit width, Pulp percentage, TSS, Peel Percentage, Fruit thickness, Stone length, Stone thickness, and Stone weight was higher in Udupi compared to Dakshina Kannada. The mean values for Leaf blade length, Leaf blade width, and Petiole length were higher in Udupi than Dakshina Kannada. The mean values for Fruit weight, Pulp percentage, Peel Percentage,

Fruit thickness, Stone thickness, and Stone weight were also higher in Udupi compared to Dakshina Kannada. In contrast, the mean values for TSS and Stone length were higher in Dakshina Kannada than Udupi. The standard deviation values for Leaf blade length, Leaf blade width, Petiole length, Fruit length, Fruit diameter, Fruit weight, Fruit width, Pulp percentage, TSS, Peel Percentage, Fruit thickness, Stone length, Stone thickness, and Stone weight were all higher in Udupi compared to Dakshina Kannada. There is a significant variability in the quantitative morphological traits of mango populations from different regions. The higher CV values for certain traits in each region indicate the potential for selective breeding efforts to improve the performance of mango populations in that region.

Table 10 : Coefficient of variation, Mean and Standard deviation of qunatitative morphological traits of leaf, petiole, fruit and stones of Dakshina Kannada and Udupi

	Udupi	Dakshina Kannada	CV Udupi	CV Dakshina Kannada
Leaf blade length (cm)	16.5 ±4.17	19.76±3.92	25.25	19.87
Leaf blade width (cm)	4.42±0.93	4.96 ±0.72	21.05	14.59
Petiole length (cm)	2.68±0.92	3.13±0.68	34.19	21.88
Fruit length (cm)	5.49±0.82	5.31±0.74	15.01	13.93
Fruit diameter (cm)	14.07±3.77	13.47±3.07	26.83	22.84
Fruit weight (g)	65.16±25.32	67.04±25.48	38.84	38.01
Fruit width (cm)	4.13±0.79	4.64±0.69	19.18	14.99
Pulp percentage (g)	12.87±6.36	17.48±11.52	49.44	65.93
Tss (Brix)	17.73±3.68	18.62±3.04	20.78	16.35
Peel percentage (%)	20.54±8.57	19.98±6.12	41.75	30.66
Fruit thickness (cm)	3.95±0.54	3.51±1.036	13.72	29.56
Stone length (cm)	5.05±0.86	4.81±0.58	17.06	12.24
Stone thickness (cm)	2.11±0.25	1.53±0.77	12.28	50.51
Stone weight (g)	29.28±11.26	27.12±9.53	38.46	35.16

The Genotypic Coefficient of Variation (GCV) is a statistical measure that describes the amount of genetic variability present in a population for a particular trait in table 11. The

GCV is calculated as the ratio of the standard deviation of the genotypic values to the mean of the same trait, expressed as a percentage. A high GCV indicates a greater amount of genetic variation in the population, while a low GCV indicates little genetic variation. It was measured several traits in a population and found that Leaf blade length, Petiole length, fruit weight, Pulp percentage, Peel percentage, fruit thickness, Stone thickness, Stone weight possess high GCV values. Leaf blade width, fruit length, fruit diameter, fruit width, TSS, and Stone length had medium GCV values, indicating a moderate level of genetic variation for these traits. While not as genetically variable as the high GCV traits, these medium GCV traits still have potential for improvement through breeding. The Phenotypic Coefficient of Variation (PCV) is another statistical measure that describes the total variation of a trait within a population, including both genetic and environmental factors. The PCV is calculated as the ratio of the standard deviation of the phenotypic values to the mean of the same trait, expressed as a percentage. In the present study leaf blade length, petiole length, fruit diameter, fruit weight, pulp percentage, peel percentage, fruit thickness, stone thickness, and stone weight had high PCV values, indicating that these traits have a significant amount of variation. in both genetic and environmental factors. Leaf blade width, fruit length, fruit width, TSS, and Stone length had medium PCV values, suggesting that they also have a moderate amount of variation in both genetic and environmental factors. The Genetic Advance as a Percentage of Mean (GAM) is a measure of the expected gain in a trait when selecting the best-performing individuals for breeding. The GAM is calculated as a percentage of the mean value for that trait in the population. The results showed that several traits had high GAM values, including Leaf blade length, Leaf blade width, Petiole length, Fruit length, Fruit diameter, Fruit weight, Fruit width, Pulp percentage, TSS, Peel percentage, Fruit thickness, Stone length, Stone thickness, and stone weight. This indicates that selecting individuals with high values for these traits will result in a significant gain in that trait in the next generation. It was found that several traits in the population had high levels of genetic variation, including Leaf blade length, Petiole length, fruit weight, Pulp percentage, Peel Percentage, fruit thickness, Stone thickness, and Stone weight. Leaf blade width, fruit length, fruit diameter, fruit width, TSS, and Stone length had moderate levels of genetic variation and could also benefit from selective breeding. By selecting individuals with high values for these traits, significant gains can be made in improving the overall performance of the population.

Table 11 : List of qualitative characters of different plant varieties of coastal districts of Karnataka

Characters	Mean	Range min\max	GCV %	PCV %	h^2%	GAM %
Leaf blade length (cm)	18.1822	8.5600 \ 25.7600	22.7077	23.3897	0.9425	45.4139
Leaf blade width (cm)	4.6982	2.1800 \ 6.5600	16.7025	18.0621	0.8551	31.8172
Petiole length (cm)	2.9129	1.0600 \ 5.0500	25.7387	28.016	0.844	48.7117
Fruit length (cm)	5.3987	3.9200 \ 7.0800	13.7927	14.1033	0.9564	27.7874
Fruit diameter (cm)	13.7669	2.3100 \ 28.7000	19.0558	24.302	0.6149	30.7808
Fruit weight (g)	66.1242	24.5450 \ 134.1037	36.6393	37.2802	0.9659	74.1794
Fruit width (cm)	4.3956	2.5400 \ 6.1300	17.0242	17.4264	0.9544	34.2606
Pulp (%)	15.2316	2.6720 \ 42.2800	60.2668	61.4047	0.9633	121.8489
TSS (brix)	18.189	11.5800 \ 25.0900	17.8338	18.0834	0.9726	36.2304
Peel (%)	20.2574	7.0440 \ 32.9200	34.8523	35.5632	0.9604	70.3603
Fruit thickness (cm)	3.724	1.7600 \ 5.5100	22.1405	22.4534	0.9723	44.9737
Stone length (cm)	4.9277	2.8300 \ 6.5600	14.2628	14.6541	0.9473	28.5967
Stone thickness (cm)	1.8129	0.3000 \ 2.6700	34.6567	34.9507	0.9832	70.7924
Stone weight (g)	28.1801	11.9750 \ 60.9371	35.3015	36.0604	0.9584	71.1908

The Table 12 shows the results of an experiment conducted to study the traits of wild mango varieties. The traits measured were leaf blade length, leaf blade width, petiole length, fruit length, fruit diameter, fruit weight, fruit width, pulp, TSS, peel percentage, fruit thickness, stone length, stone thickness, and stone weight. The experiment was conducted with two replicates and three treatments. The analysis of variance (ANOVA) for each trait revealed that leaf blade length, leaf blade width, and peel percentage had significant variation in both replicates and treatments. The remaining traits, including petiole length, fruit length, fruit diameter, fruit weight, fruit width, pulp, TSS, fruit thickness, stone length, stone thickness, and stone weight did not show any significant variation in either replicates or treatments.

In the table 12, shows Leaf Blade Length, Leaf Blade width, Peel Percentage has significant variation both replicate and treatments.But remaining traits does not posess any significant variation in replications not in treatment as value referred > 0.5.

Table 12: Analysis of variation in plant characteristics across replication and treatments.

Source of varitation	**Leaf blade length**	**Leaf blade width**	**Petiole length**	**Fruit length**
Replications	2.0029\ 0.0378	1.9859 \ 0.0396	0.8819 \ 0.5414	1.8839 \ 0.0528
Treatments	17.4009 \ 0.00	6.9021 \ 0.0	6.4116 \ 0.0	22.9600 \ 0.00
Error				

Source of varitation	**Fruit diameter**	**Fruit weight**	**Fruit width**	**Pulp percentage**	**TSS**
Replications	1.0591 \ 0.3923	1.4149 \ 0.1794	1.6412 \ 0.1016	0.5241 \ 0.8172	0.7051 \ 0.7044
Treatements	2.5964 \ 0.0	2.3361 \ 0.00	21.9172 \ 0.00	27.2329 \ 0.00	36.4708 \ 0.00
Error					

Source of varitation	**Peel percentage**	**Fruit thickness**	**Stone length**	**Stone thickness**	**Stone weight**
Replications	2.4476 \ 0.0101	1.9008 \ 0.0504	1.8267 \ 0.0618	1.6646 \ 0.0956	1.6265 \ 0.1056
Treatements	25.2624 \ 0.00	36.1284 \ 0.00	18.9762 \ 0.00	59.6993 \ 0.00	24.0122 \ 0.00
Error					

4.0 RESULTS

The results obtained from the present investigation "Evaluation of genetic variability of wild mango varieties of Dakshina Kannada and Udupi Districts of Karnataka using molecular markers"are presented under the following details.

1.Isolation of plant genomic DNA and quantification

2.Analysis of wild mango varieties using SSR primers

The DNA isolated from the leaves of 42 mango varieties subjected to quantification and also described in Table 1.

Table 1: Quantification of DNA of different varieties of Dakshina Kannada and Udupi districts

Sl.No.	Variety	DNA (ng/µL)	$A_{260/280}$	$A_{260/230}$
1	Kelakila	75.5	2.67	2.89
2	Hebri	36.7	1.6	18.23
3	Adishakthi mudradi	52.8	1.9	1.255
4	Mudradi	93.8	2.0	9.18
5	Bailumane-I	52.8	1.9	3.85
6	Sitanadi	141.5	2.0	1.7
7	Muniyal	54.3	1.7	3.4
8	Bailumane 2	202.4	2.0	3.195
9	Sitanadi	118.1	2.0	2.1
10	Yennehole	238.0	1.8	4.5
11	Jodurasthe	243.4	2.5	0.3
12	Jodukatte	35.1	2.3	0.052
13	Anekere	90.9	2.4	3.0
14	Karkala bypass	18.1	2.1	0.2
15	Pulkeri	2019.8	4.2	0.96
16	Miyar kuntibail	195.0	2.1	0.0
17	Borugudde	35.2	2.03	32.06
18	Bannadka	40.3	2.09	0
19	Beluvayi	73.3	1.9	9.554
20	Sampige	2361	2.8	1.3

21	Mastikatte	13.4	1.7	0.39
22	Thacode 1	232.5	1.7	0.655
23	Thacode 2	560.9	3.5	0.97
24	Meginamane	44.0	2.120	0
25	Alangar	280	2.07	4.03
26	Birav	64.2	2.2	0.91
27	Paper mill	59.4	2.10	0
28	Ontikatte	599.9	2.08	2.7
29	Laadi	301.10	2.25	2.180
30	Kodiyadka	90.3	1.9	2.6
31	Koragatte	141.5	2.10	7.23
32	Dhande	8.0	2.9	0
33	Kodiyadka	27.5	1.9	0
34	Siddakatte	67.5	0.8	0.3
35	Karimale	76.5	2.0	3.3
36	Ananthady	122.7	2.06	6.43
37	Nelyakumar	152.3	2.0	4.9
38	Korey	75.5	2.09	9.807
39	Kuthkoli	40.3	1.85	24.7
40	Padil	50.2	2.1	0.11
41	Irvaje	391.2	2.04	2.93
42	Pilimogaru	255.3	2.11	3.12

The genetic relationships among mango accessions were examined using SSR markers and results are represented in Table 2. A total of 180 alleles and 22.50 alleles per locus were examined across all mango varieties using primers. The observed heterozygosity (H_O) ranged from 0.15 to 0.63 with an average of 0.41 while the expected heterozygosity (H_E) varied from 0.57 to 0.95 with a mean of 0.83.

Table 2: Molecular data showing observed heterozygosity, expected heterozygosity, polymorphic information content for various genotypes using different SSR primers

Sl. No	Primer Name	Primers size		No. of alleles	Ho	He	PIC value
		Forward (5'-3')	Reverse (5'-3')				
1	11588	GTAAACGACGGCCAGTGAGAAATGGTTCCAGCAA	GTTCTTCACGCGAAGTAAACCAAA	25	0.6053	0.9147	0.8962
2	8095	GTAAAACGACGGCCAGTTGAATGGGTAATGGTGAATCG	GTTTCTTTCAACTCAATCTGATCGCTTACA	16	0.1500	0.5728	0.555
3	333	GTAAAACGACGGCCAGTAAACCATTGTGGATGTGGGT	GTTTCTTTCCCAGTCTGGAAAAAGAAAAA	26	0.4146	0.8943	0.8763
4	MiIIHR99	GTAAAACGACGGCCAGTCTTCATCGAATCCAAGGCAT	GTTTCTTCTTCCATGGCACGAGTAGGT	28	0.6341	0.9085	0.8902
5	21478	GTAAAACGACGGCCAGTAAACTTTAATGGATTTTATCGTGTAAT	GTTTCTTAAATACCCCACCCATTTTGC	21	0.3333	0.7855	0.7657
6	16400	GTAAAACGACGGCCAGTAAATCCCGTACCTTCATCCC	GTTTCTTTGCCAGAACTGCTCTCTTCA	16	0.3415	0.6865	0.6654
7	MiKVR80	GTAAAACGACGGCCAGTTACAGGCTGCCCAGAAAGAT	GTTTCTTGCGCATGCTGGGATTAGTAT	30	0.2727	0.9077	0.8859
8	MiIIHR17	GCTTGCTTCCAACTGAGACC	GCAAAATGCTCGGAGAAGAC	30	0.5641	0.9491	0.9336

The data associated with the assessment of the genetic diversity of 42 mango varieties collected in the present study mentioned in figure 2.

Cluster 1: Kelakila, Thacode 1, Laadi, and Pilimogaru. This cluster indicates a high level of genetic diversity within the group, suggesting that these varieties share a common genetic background but also exhibit considerable genetic variation.Cluster 2:Hebri, Adishakthi mudradi, Mudradi, Bailumane-1, Sitanadi, Sitanadi,Yennehole, Jodurasthe, Miyar kuntibail,

Sampige, Thacode 2, Alangar,Birav, Ontikatte, Dhande, Kodiyadka, Siddakatte, Nelyakumar.

Cluster 3: Muniyal, Koragatte. These two varieties are closely related to each other but distinct from the other clusters. Cluster 4: Bailumane 2, Jodukatte, Anekere, Pulkeri, Borugudde, Bannadka, Beluvayi, Meginamane, Papermill, Kodiyadka, Karimale, Ananthady, korey, Kuthkoli, Padil. Cluster 5: Masthikatte, Karkala bypass and it represents another significant group of genetically similar varieties, indicating a shared lineage among these mango varieties.

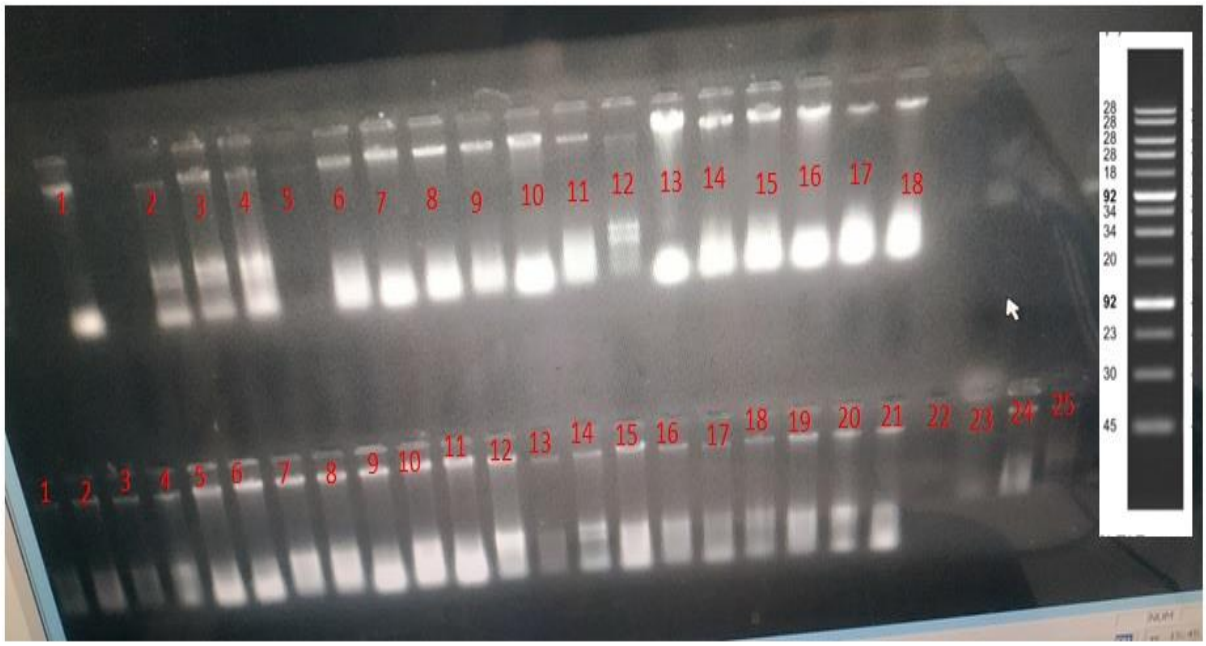

Figure 1: Profiles of amplified PCR products produced by SSR markers

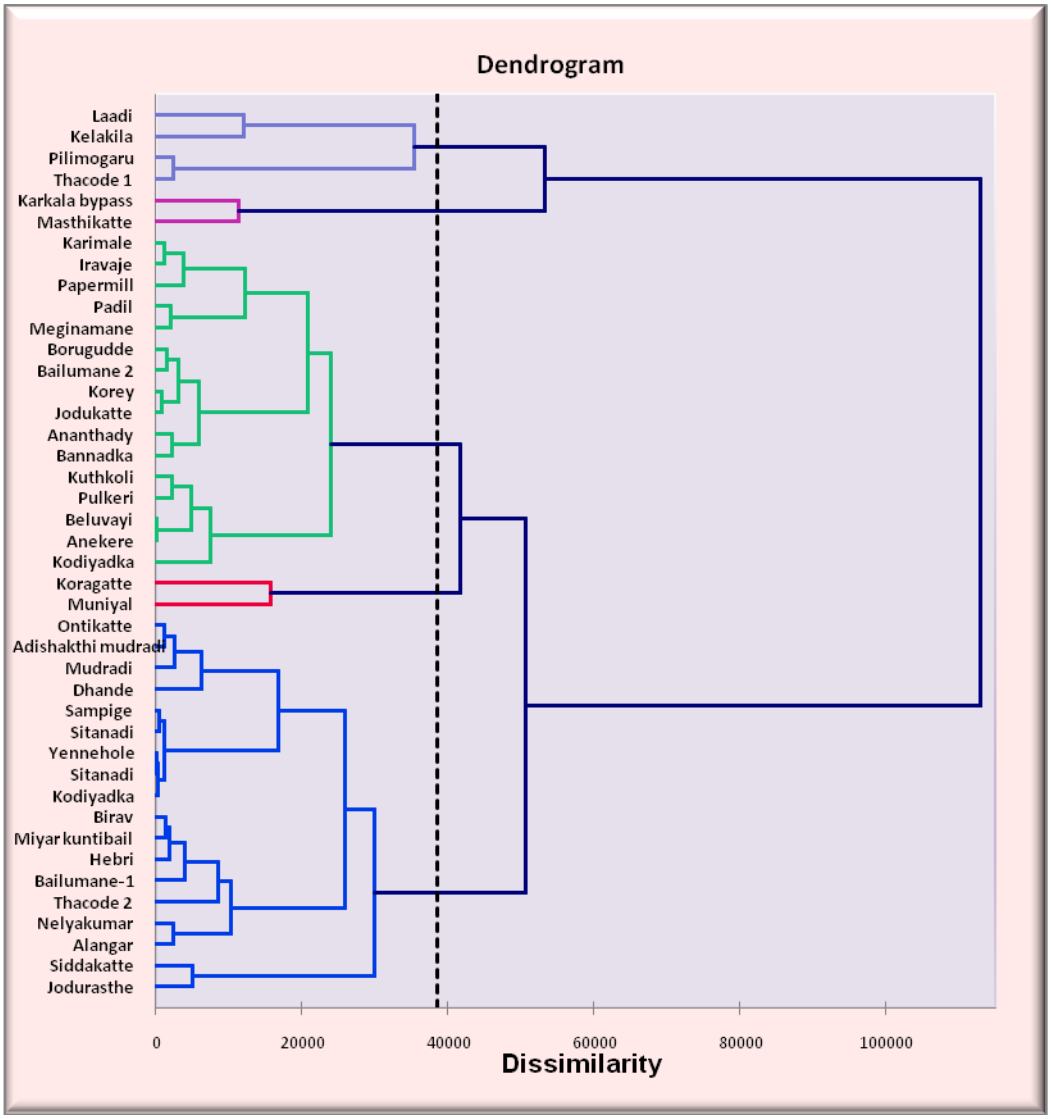

Figure 2: Molecular data representing the relationship among 42 mango varieties in Dendrogram

The assessment of genetic variation of wild mangoes there by clustering showing cluster groups in Table 3.

Objects: There are number of objects in this cluster.

Sum of weights: The total weight of the objects in the cluster.

Within-class variance: This measures how much the data points within the cluster differ from the cluster's centroid. A higher value suggests more variability within the cluster.

Minimum distance to centroid: The minimum distance of any object in the cluster to its centroid.

Average distance to centroid: The average distance of all objects in the cluster to its centroid.

Maximum distance to centroid: The maximum distance of any object in the cluster to its centroid.

The clustering analysis indicates significant genetic diversity among the mango varieties. Cluster 2, with 18 objects, shows the lowest within-class variance and a broad range of distances to the centroid, reflecting high internal diversity. In contrast, Clusters 3 and 5, each with only 2 objects, exhibit higher within-class variance, suggesting a more distinct separation from other clusters. Cluster 1, with its higher within-class variance and substantial maximum distance to the centroid, highlights significant internal variability.

Table 3: Molecular data showing the cluster results of wild mango varieties.

Cluster Groups					
Class	1	2	3	4	5
Objects	4	18	2	16	2
Sum of weights	4	18	2	16	2
Within-class variance	16609.250	6936.971	15741.000	6134.304	11322.000
Minimum distance to centroid	98.645	36.740	88.716	40.950	75.240
Average distance to centroid	110.452	74.179	88.716	72.184	75.240
Maximum distance to centroid	138.070	147.309	88.716	122.345	75.240
	Kelakila	Hebri	Muniyal	Bailumane 2	Masthikatte
	Thacode 1	Adishakthi mudradi	Koragatte	Jodukatte	Karkala bypass
	Laadi	Mudradi		Anekere	
	Pilimogaru	Bailumane-1		Iravaje	
		Sitanadi		Pulkeri	
		Sitanadi		Borugudde	
		Yennehole		Bannadka	
		Jodurasthe		Beluvayi	
		Miyar kuntibail		Meginamane	
		Sampige		Papermill	
		Thacode 2		Kodiyadka	
		Alangar		Karimale	
		Birav		Ananthady	

		Ontikatte		Korey	
		Dhande		Kuthkoli	
		Kodiyadka		Padil	
		Siddakatte			
		Nelyakumar			

Eigen values represent the amount of variation captured by each principal component. Higher eigenvalues indicate that the corresponding principal component explains a larger proportion of the total variance in the data. Lower eigenvalues indicate that the corresponding principal component explains in Table 4 a smaller proportion of the total variance.

Table 4: Eigen values by axis and sample eigen vectors for wild mango varieties

Axis No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
EigenValue	5.981	4.823	4.271	3.706	3.549	3.199	2.932	2.799	2.523	2.202
Kelakila	-0.086	0.336	0.380	-0.049	0.713	-0.098	0.**216**	0.291	0.173	-0.171
Hebri	0.143	0.413	-0.522	0.007	-0.103	0.259	0.335	-0.014	-0.205	-0.004
Adishakthi mudradi	0.131	-0.074	-0.361	0.231	0.583	0.057	0.327	-0.023	0.163	-0.456
Mudradi	0.099	-0.023	-0.473	-0.085	0.205	0.347	0.169	0.273	-0.093	-0.172
Bailumane-1	-0.084	-0.404	-0.261	0.574	0.108	0.474	-0.190	0.401	0.105	-0.001
Sitanadi	-0.129	-0.475	0.045	0.345	0.**408**	-0.518	-0.040	0.070	-0.449	0.166
Muniyal	-0.408	0 .137	-0.097	0.277	-0.031	-0.322	-0.169	-0.224	0.145	0.015
Bailumane 2	-0.096	0.017	-0.458	-0.459	-0.004	0.131	-0.193	-0.125	-0.068	0.094
Sitanadi	-0.493	-0.213	-0.474	0.208	-0.252	0.328	-0.069	0.125	0.107	0.113
Yennehole	-0.505	-0.445	-0.138	-0.026	-0.380	0.164	0.147	-0.200	0.029	0.109
Jodurasthe	-0.519	-0.112	0.520	-0.132	-0.136	0.225	0.384	0.231	0.132	-0.071
Jodukatte	-0.445	-0.339	-0.012	0.174	-0.463	0.188	0.019	-0.146	0.251	0.014
Anekere	-0.170	-0.337	0.011	-0.083	-0.314	-0.201	-0.090	0.175	-0.224	-0.024
Iravaje	0.081	-0.352	0.146	0.072	0.045	-0.003	-0.513	-0.027	0.054	-0.317
Pulkeri	0.627	-0.282	0.370	0.043	-0.250	0.132	0.446	-0.559	-0.373	-0.291
Miyar kuntibail	0.427	-0.086	0.140	0.259	-0.023	0.034	-0.033	-0.126	-0.556	0.007
Borugudde	0.068	-0.816	0.421	0.164	0.080	0.051	0.288	-0.143	-0.251	0.165
Bannadka	0.059	-0.204	-0.250	-0.375	-0.113	-0.490	0.310	0.208	-0.582	0.217

Beluvayi	0.764	0.164	-0.070	-0.002	-0.408	-0.624	-0.116	0.441	-0.052	-0.323
Sampige	-0.165	0.188	-0.108	0.553	-0.272	-0.207	0.294	-0.372	0.347	0.105
Thacode 1	-0.617	0.144	0.350	-0.230	-0.109	0.175	-0.324	0.177	-0.147	-0.140
Thacode 2	-0.344	0.168	-0.260	-0.424	-0.087	-0.047	-0.235	-0.118	0.009	0.038
Meginamane	-0.637	0.196	0.135	0.144	0.283	-0.214	-0.057	0.007	0.033	-0.182
Alangar	0.272	0.328	0.126	0.361	0.442	-0.120	-0.349	-0.557	0.078	0.161
Birav	-0.116	-0.313	0.079	0.258	0.358	-0.101	-0.369	0.034	0.138	0.016
Papermill	0.068	-0.234	0.515	-0.402	0.106	0.288	0.072	-0.232	0.140	-0.625
Ontikatte	-0.217	-0.114	-0.204	-0.325	-0.046	-0.060	0.271	-0.480	0.176	-0.083
Laadi	-0.106	0.934	0.522	-0.306	-0.051	0.251	0.156	0.189	-0.240	0.164
Kodiyadka	-0.146	0.163	-0.285	-0.104	0.165	-0.605	0.360	0.067	0.162	0.135
Masthikatte	0.194	0.419	-0.221	-0.019	-0.395	0.027	-0.043	-0.168	0.165	0.079
Koragatte	0.426	-0.384	0.152	-0.355	0.310	0.004	0.065	0.475	0.385	0.566
Pilimogaru	-0.220	0.397	0.588	0.263	-0.314	0.228	-0.218	0.255	-0.313	0.133
Dhande	-0.066	0.475	-0.417	0.039	0.350	0.007	0.212	-0.095	0.052	0.007
Kodiyadka	-0.218	-0.121	-0.430	-0.572	-0.004	-0.047	-0.197	-0.191	-0.197	0.157
Siddakatte	-0.469	0.526	0.345	0.511	-0.039	-0.287	0.150	0.027	-0.081	0.109
Karimale	0.508	0.282	-0.128	0.041	-0.601	-0.349	-0.289	0.200	0.244	-0.486
Ananthady	0.268	-0.252	0.020	0.236	-0.329	0.021	-0.175	0.332	0.274	0.182
Nelyakumar	0.646	0.258	0.236	-0.118	0.103	0.200	-0.548	-0.519	0.038	0.451
Korey	0.212	-0.221	-0.113	-0.333	0.360	-0.065	-0.466	0.019	-0.076	-0.185

Kuthkoli	0.801	-0.175	0.394	-0.173	-0.144	-0.062	0.386	0.030	0.573	0.287
Padil	-0.203	0.114	0.218	-0.556	0.119	0.131	-0.033	0.073	0.172	0.002
Karkala bypass	0.665	0.318	-0.431	0.365	0.132	0.697	0.109	0.220	-0.235	0.038

The first principal component (Axis 1) has the highest eigenvalue of 5.981.This suggests that Axis 1 explains in the most variability in the dataset, capturing approximately 5.981 units of variance. Subsequent principal components have decreasing eigenvalues, indicating decreasing amounts of explained variance. Generally, eigenvalues greater than 1 are considered significant in PCA. In this dataset, all eigenvalues are greater than 1, indicating that each principal component explains a substantial amount of variance in the data.

Eigen values help in determining the number of meaningful principal components to retain in the analysis. Components with eigenvalues significantly greater than 1 are typically retained, as they capture substantial variance in the data. The eigenvalues provide insight into the relative importance of each principal component in explaining the variability present in the dataset. Higher eigenvalues suggest greater importance in capturing variation, aiding in the interpretation and dimensionality reduction of the data through techniques like PCA.

Genetic diversity among populations

The analysis reveals a moderate level of genetic diversity among populations, with a Shannon diversity index (Shannon) estimated at 0.267 given in table 5. Approximately 11.076% of the total genetic diversity observed in the dataset is attributed to differences among populations.

Table 5: Shannon Statistics for among the population and within populations for wild mango varieties

Source of Information	**Degrees Freedom**	**Log-Like. Chi-Sq**	**Shannon Inform.**	**Percent of Total**	**Diversity Estimate**	**[0,1] Scaled Diversity**	**[0,1] Scaled Overlap**	**Estimated Probability**
	DF	**G-Test**	**sH**	**Inform.**	**exp(sH)**	**D'**	**O'=1-D'**	**P(rand >= data)**
Among Pops	1	41.461	0.267	11.076	1.306	0.469	0.531	0.058

Within Pops	80	333.524	2.143	88.924	8.525	0.906	0.094	0.943
Total	81	374.985	2.410	100.000	11.134	0.922	0.078	

Within populations, the level of genetic diversity is substantially higher, with a Shannon diversity index estimated at 2.143 represented in figure 2. The majority of the genetic diversity, approximately 88.924%, is found within populations. The total genetic diversity observed in the dataset, as measured by Shannon's information index, is estimated at 2,410. Among the total genetic diversity, 11.134% is attributed to among-population differences, while the remaining 88.866% is within-population diversity.

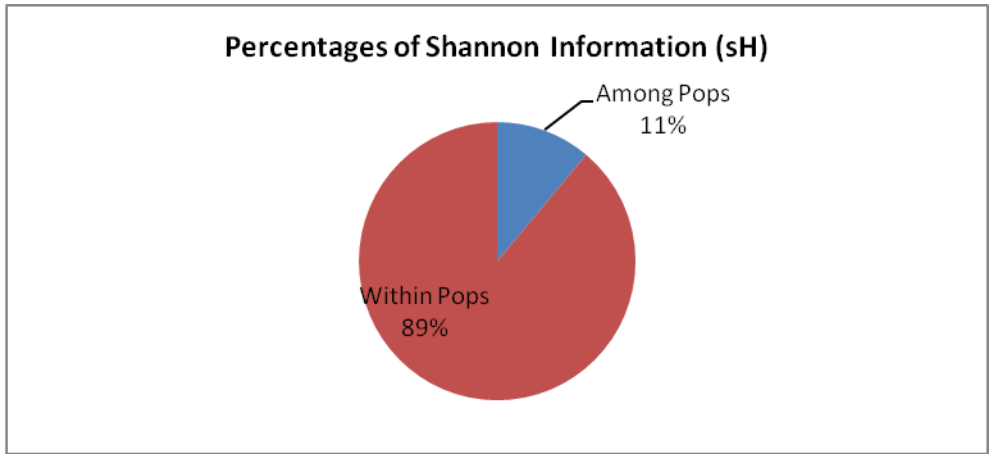

Figure 2: Shannon informational diversity statistics for wild mango varieties

The significant contribution of within-population genetic diversity to the overall genetic variation observed in the studied populations. These underscore the importance of considering both among-population and within-population diversity.

The Chi-Square tests were conducted to assess the Hardy-Weinberg Equilibrium (HWE) for genetic loci within two populations, Udupi and DK, across 8 different loci represented in Table 6. A total of 42 samples were analyzed, with each population contributing to the dataset.

Table 6. Hardy-Weinberg Equilibrium for genetic loci within populations of Dakshina Kannada and Udupi

Populations	**Locus**	**DF**	**ChiSq**	**Prob**	**Significant**
Udupi	SSR1	171	325.000	0.000	***
Udupi	SSR2	78	200.045	0.000	***
Udupi	SSR3	21	107.993	0.000	***
Udupi	SSR4	276	378.889	0.000	***
Udupi	SSR5	120	188.265	0.000	***
Udupi	SSR6	36	103.697	0.000	***
Udupi	SSR7	136	220.142	0.000	***
Udupi	SSR8	78	197.416	0.000	***
DK	SSR1	91	152.899	0.000	***
DK	SSR2	36	51.479	0.046	*
DK	SSR3	36	120.139	0.000	***
DK	SSR4	36	89.542	0.000	***
DK	SSR5	136	206.800	0.000	***
DK	SSR6	120	217.500	0.000	***
DK	SSR7	231	348.833	0.000	***
DK	SSR8	66	150.000	0.000	***

Key: Ns =not significant, * P<0.05, ** P<0.01, *** P<0.001

For the Udupi population, significant deviations from Hardy-Weinberg Equilibrium (HWE) were observed across all loci (SSR1-SSR8). Chi-Square test statistics ranged from 103.697 to 378.889, with degrees of freedom ranging from 21 to 276, indicating highly significant departures from HWE. The degrees of freedom (DF) varied across loci, reflecting the different genetic characteristics and allele frequencies at each locus within the Udupi population.

The Dakshina Kannada population exhibited significant deviations from HWE across all loci (SSR1-SSR8), except for SSR2. Significant Chi-Square test statistics were observed, ranging from 51.479 to 348.833, with degrees of freedom ranging from 36 to 231, indicating highly

significant departures from HWE. SSR2 was the only locus where the deviation from HWE was not statistically significant (Prob = 0.046), although it still demonstrated a notable departure.Across both populations and all loci, the majority of the deviations from HWE were highly significant (*** P <0.001). One locus in the DK population (SSR2) showed significance at the * P<0.05 level, indicating a less pronounced but still notable departure from HWE. The departure from HWE at SSR2, although statistically significant, is less pronounced compared to other loci. This indicates potential differences in the genetic structure or evolutionary dynamics of the DK population compared to the Udupi population.

PCA Analysis has been conducted for 42 samples collected from different locations here this statistical technique represented in figure 3 used to reduce the dimensionality of data while retaining as much information as possible. From the provided PCA results eigenvalues represent the amount of variance explained by each principle component here F1 represents the value 2.105, F2 represents 1.813, F3 represents 1.105 and so on and better explained in terms of variability (%) F1 explains 26.309% of the total variance, F2 explains 22.667% of the total variance. F3 explains 13.818% of the total variance and these percentages help understand the relative importance of each principal component in explaining the variance in the data. Here the first two principal components (F1 and F2) together explain 48.976% of the total variance. And these two components used for plotting the PCA biplot and only these two PC values are enough to retain a sufficient number of PC to explain most of the variance in the data while reducing dimensionality.

The scatter plot of observations on the first two principal components (F1 and F2) shows how different observations (locations) are positioned in the space defined by these components. The axes represent the principal components, with F1 accounting for 26.31% and F2 for 22.67% of the total variance, together explaining about 48.98% of the variance in the data.

From the PCA Biplot F1 (Component 1) explains 26.31% of the variance in the data, and F2 (Component 2) explains 22.67% of the variance. Together, they account for 48.98% of the total variance.Each red dot represents an observation in the dataset.The blue labels indicate the names of the observations.The plot shows how the observations are spread out in the new feature space. Observations close to each other are similar in terms of the variables included in the analysis. Points that are far from the center or isolated from others (like "Karkala bypass" and

"Kelakila") may be considered outliers. Kelakila and Muniyal are particularly important for F1. Kelakila and Jodurasthe are significant for F2. F1 (26.31% variance): Key observations are Kelakila, Muniyal, and Thacode 1. F2 (22.67% variance): Influential observations include Kelakila and Jodurasthe.

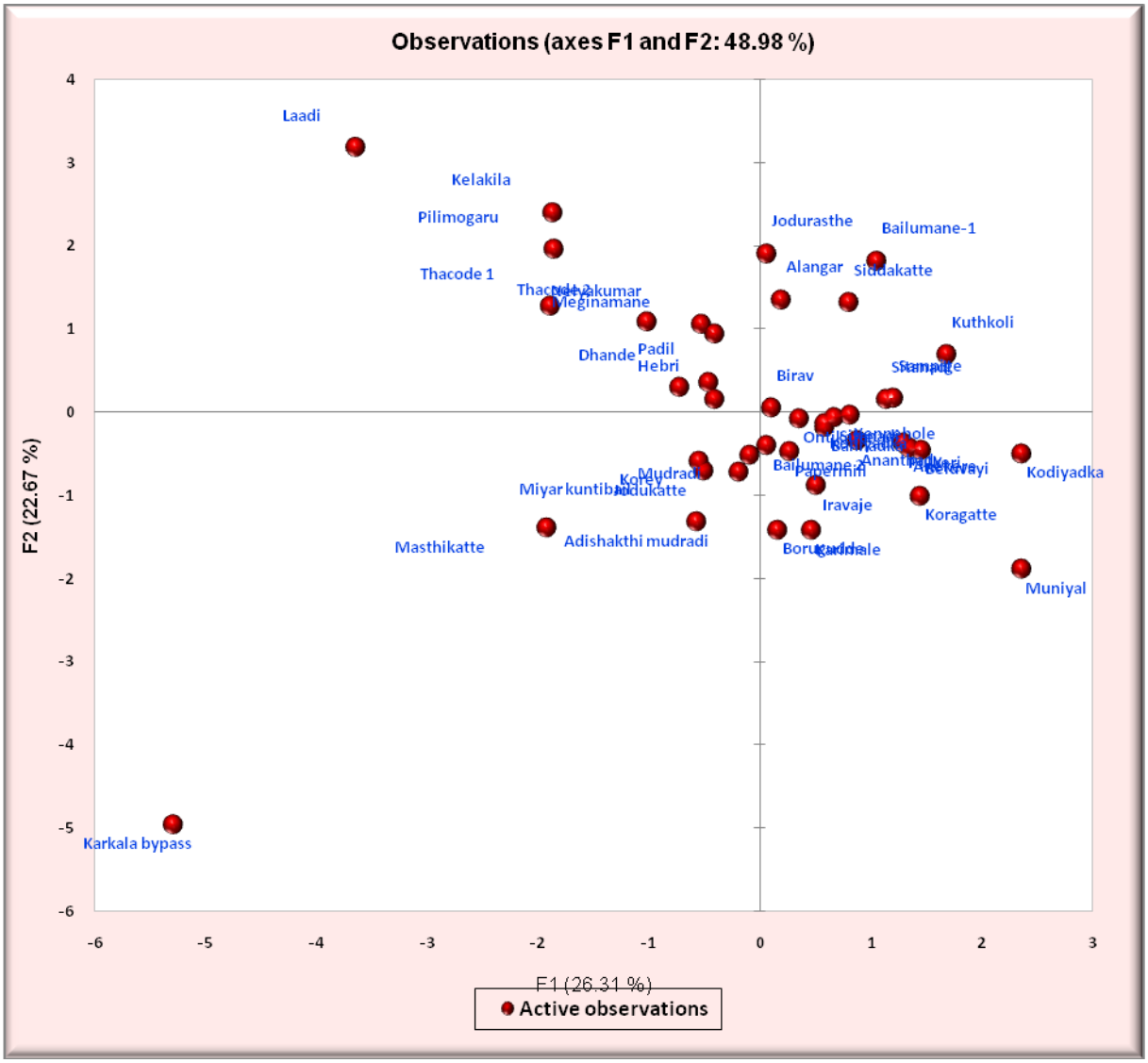

Figure 3: Principal Component Analysis of 42 wild mango varieties of Dakshina Kannada and Udupi districts

Principal Coordinate Analysis (PCoA), also known as Metric Multidimensional Scaling (MDS), is a technique used to visualize the dissimilarities of data. PCoA is typically used for data where the dissimilarity (distance) between observations is of primary interest represented in figure 4. PCoA starts with a distance matrix, which represents the pairwise distances between observations. Here Euclidean distance is used for the calculation, PCoA

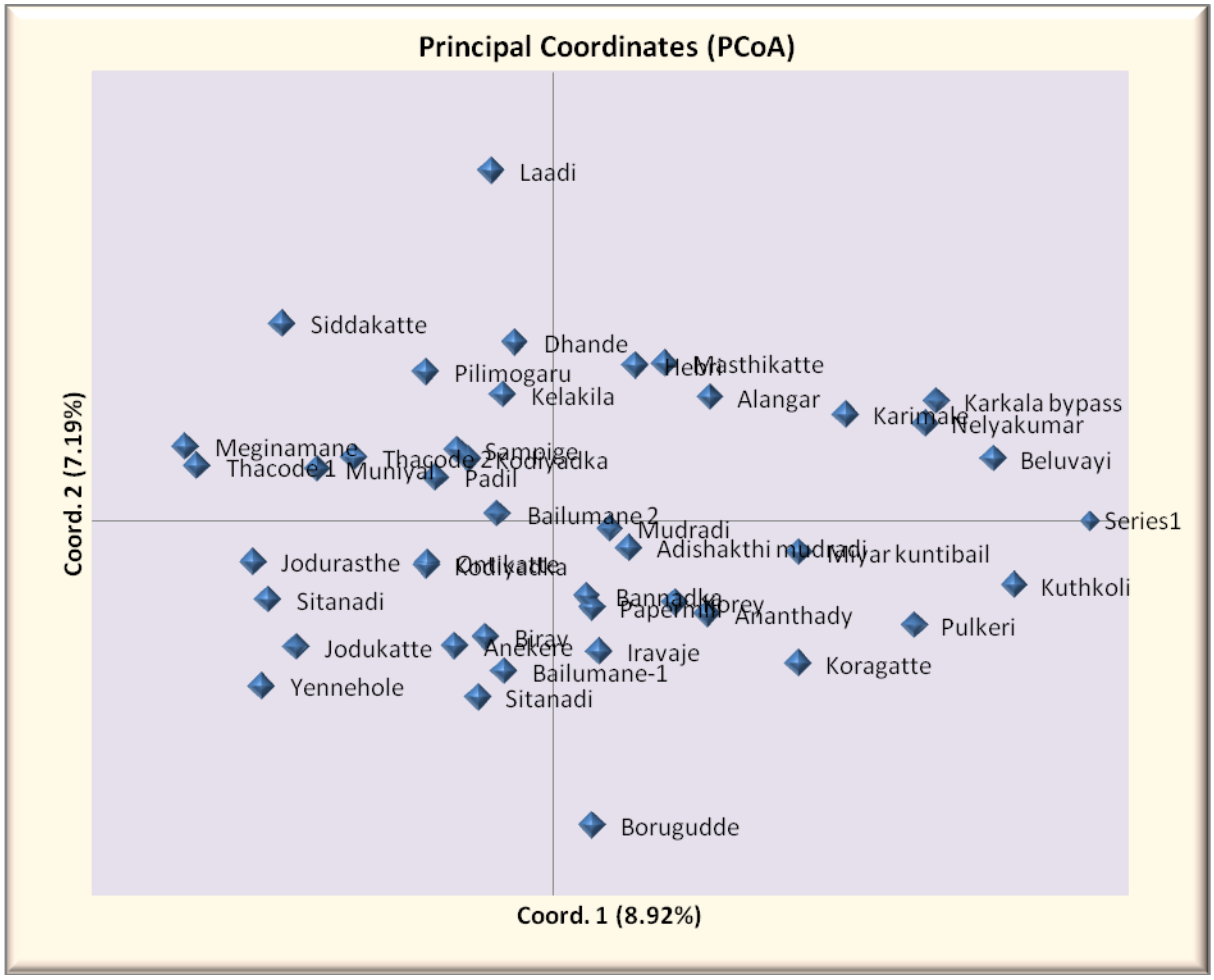

Figure 4 :Principal coordinate analysis of 42 wild varieties of mangoes of Dakshina Kannada and Udupi districts

involves eigenvalue decomposition but applied to the distance matrix. The goal is to find a set of coordinates (principal coordinates) that best represent the distances between observations in a lower-dimensional space.

Here, PcoA Biplot derived from the data of genetic distance. Here the plot displays the first two principal coordinates (Coord. 1 and Coord. 2), which capture the largest amount of

variance in the data.Coord. 1 explains 8.92% of the variance and Coord. 2 explains 7.19% of the variance. Together, these two coordinates explain 16.11% of the total variance in the dataset. A large number of observations are clustered around the center, indicating they have similar characteristics. Examples include Sitanadi, Bailumane-1, and Iravaje.

Some points like Laadi and Borugudde are located on the periphery, indicating they are quite distinct from the central cluster and each other. Positioned between the central cluster and periphery, indicating they have some unique attributes but also share commonalities with the central group. The first two coordinates explain a relatively small portion of the total variance (16.11%), suggesting that more dimensions (beyond Coord. 1 and Coord. 2) are needed to fully capture the complexity of the data.

This table 6 summarizes the results of an Analysis of Molecular Variance (AMOVA) test. AMOVA is used to partition genetic variation within and among populations based on molecular marker data. The table 7 has four rows and six columns with headings for Source of Variation, Degrees of Freedom (df), Sum of Squares (SS), Mean Squares (MS), Estimated Variance, and Percentage of Variation.

Table 7 : Percentage of molecular variance of wild mango varieties of Udupi and Dakshina Kannada

Source	**Df**	**SS**	**MS**	**Est. Var.**	**%**
Among Pops	1	15.009	15.009	0.222	2%
Within Pops	40	414.182	10.355	10.355	98%
Total	41	429.190		10.577	100%

The first row shows results for variation Among Populations. It indicates there is 1 df, an SS of 15.009, MS of 15.009, estimated variance of 0.222, accounting for 2% of total variation. The second row displays results for variation Within Populations. It lists 40 df, SS of 414.182, MS of 10.355, estimated variance of 10.355, accounting for 98% of total variation. The third row totals the previous rows. It gives 41 df, SS of 429.190, and indicates this totals to 100% of genetic variation. This AMOVA results table partitions genetic variation found in molecular marker data into two main components - variation among tested populations vs. within populations. It shows

most (98%) of the genetic variation exists within populations ratherthanamong populations (2%). This quantifies population structure and genetic differentiation.

5.DISCUSSION

A wide variation is found in the morphological characteristics of wild mango varieties. The qualitative and quantitative characteristics of 45 wild varieties of*Mangifera indica* were evaluated and it is revealed that these varieties shows variation in shapes and sizes of leaves and fruits and with remaining parameteres and it is differ genotype to genotype. A majority of the varieties were characteristically small in size. The size and shape of a plant are determined by the density of planting, training and pruning, and cultural practices (Neto, 2000). The noticed traits are also similar to earlier reports (Selvan *et al.* 2010; Vieccelli *et* al. 2016; Igbari *et al*. 2019).

In the present study variability was observed in height of the tree, crown shape, leaf blade shape and leaf apex shape. Similar findings were found by Fivaz (2008). The majority of varieties were medium in height with 20 varieties falling in this category. However, there were also a significant number of varieties that were tall (16 varieties) and very tall (9 varieties). These findings are consistent with the natural variation observed in plant species because tree height can vary greatly depending on environmental conditions and genetic factors. (Dinesh *et al.* 2013; Singh *et al*. 2017; Sridhar *et al*. 2022). These findings are consistent with the natural

variation observed in plant species because tree height can vary greatly depending on environmental conditions and genetic factors.

The crown shape of the different plant varieties was investigated and a variety of shapescrown shape of the different plant varieties was investigated and a variety of shapes, including semicircular (13 varieties), spherical (7 varieties), circular (7 varieties), broadly pyramidal (9 varieties), oblong (8 varieties), and erect (1 variety) were found. This suggests that plant species exhibit a diverse range of crown shapes, which may be influenced by this suggests that plant species exhibit a diverse range of crown shapes, which may be influenced by genetic factors as well as environmental factors such as wind and light exposure, (Tekalign *et al*. 2017).

The majority of varieties exhibiting acuminate leaf apex shape and acute leaf base shape. However, there were also some varieties that exhibited obtuse leaf apex shape, Acuminate, acute, obtuse while one variety had round leaf base shape and similar results reported by (Ribeiro *et al*. 2013). The plant species exhibit a wide range of leaf shapes which may play a role in their adaptation to specific environmental conditions. Khan *et al*. (2016), which reported significant differences in leaf length and width among different mango varieties. Such variations in leaf morphology are important for the growth and development of mango trees, as they influence the light interception and photosynthetic efficiency of the leaves. Leaves exhibits the mild fragrance, while some of varieties has no fragrance.

The plant species show a wide range of variations in fruit shape, color, and pulp color as well as other plant traits such as tree height, crown shape, leaf apex shape, leaf base shape, and leaf fragrance. These observations highlight the importance of preserving the natural genetic diversity of plant species which may have significant implications for their adaptation to changing environmental conditions.

The most common fruit shape observed in the current study was round with 32 varieties exhibiting this shape (Rajwana *et al*. 2011; Vieccelli *et al*. 2016). The fruit shape of the varieties exhibited a different shapes including round, elliptic, ovoid, oblong, and obtuse. The most common fruit shape observed in the study was round with 32 varieties exhibiting this shape these observations were comparable with the findings earlier studies(Fivaz, 2008; Litz 2009; Ribeiro *etal*., 2013; Kheshin *et* al. 2016; Vieccelli *et* al. 2016). The relationships between fruit length and shape could be associated with the effect of length on the shape of different mango varieties as most mango posess ovate fruit shape with different modifications (Ritu *et al*. 2013). Giri *et al*.

(2018) on mango germplasm accessions in India found a wide range of fruit shapes, including round, oval, elliptical, and kidney-shaped, while fruit size varied from small to large. The slope of the fruit ventral shoulder ended in a long curve, raising and then curved and slopping abruptly. In the apical portion, a perceptible and pointed beak was noticed as well as sinus in ten cultivars noticed shallow and rest of the cultivar shown absent.

Skin color of the maximum number of mango 16 varieties were green. The other prevalent skin colors were yellowish-green and greenish-yellow. Other skin colors observed in the present study were pale yellowish, greenish pale yellow, and yellowish-orange. Similar observations were reported in earlier studies too and some of that (Mukharjee 1972; Barholia and Sennhenn *et al*. 2014; Yadav 2014). The fruit colour at maturity was dependent on genotype. Different fruit color at maturity depends on genotypes and similar to present work Saha and Hossain 1988; Haque *et al.*1993; Saha *et* al. 1994; Mukherjee 1997; Hossain *et al*. 2002 and Kobra 2007;Barholia and Yadav 2014).

The fruit apex which is usually roundish in shape. The study also reported a range of pulp colors among different mango varieties with orange being the most common color followed by pale yellow, whitish-yellow pulp color, an orange, yellow, yellow orange, pale orange, orange yellow. These variations might be due to the location enjoying different types of enviornmental conditions. Similar oservations were reported in Saha *et al*. (1994), Hossain *et al*.(2002) and Kobra (2007), Singh *et al*. (1997). Aguoru *et al.*(2016) reported that the most significant characters to influence morphological variability among mangoes are the genotypic variations.(Mithra 2016; Krishnapillai and Wijeratnam 2016; Majumder 2011).

These varieties are commonly called by different names and used for processing in to pickles where in whole fruits are pickled without dicing. These varieties of mangoes are widely grown in the Western Ghats region. All the varieties of mangoes collected from the different locations exhibited green colour with smooth texture before maturity. (Nalini and Chimmad 2005). The morphological markers and isozymes techniques in the characterization of mango cultivars often does not provide an accurate evaluation of diversity among mango cultivars, and this could lead to misidentification or duplication of the crop genotypes (Kumar *et al*., Citation 2013).

All the varieties of mangoes were sour, fragrant and acceptable for consumption at physiologically mature stage. (Nalini & chimmad 2005). Rajan *et al*. (2001) reported significant

difference in canopy structure among 26 Indian mango cultivars. Rajan (2019) reported that wild species of mango cannot overempasised as the species is in danger of loss in its natural habitat. Generally morphological markers are tree height, bearing habit, flowering pattern, inflorescence shape, colour and type of flowers, fruit shape, colour of the fruit, shoulder position, etc. (Bhat *et al*. 2010).

The morphological characteristics of 45 mango genotypes such as leaf blade length, leaf blade width, petiole length, fruit length, fruit diameter, fruit width, peel, fruit thickness, stone length, and stone thickness did not differ significantly between the genotypes. Significant differences were found between the different wild mango genotypes in their stone weight, fruit weight, pulp percentage, and TSS and higher stone weight with lower yield but pulp parcentage and TSS found to be more. In some of earlier studies there were significant variation found in parameters such as fruit weight, pulp percentage, and TSS (Ribeiro *et al*. 2013; Choudhury *et al*. 2017; Prasad *et al*. 2019). These reports suggest that the parameters identified in the present study as significant for wild mango genotypes are also important factors to consider in cultivated mango varieties. The studies have also shown that the variation in these parameters is influenced by genetic factors, environmental factors, and their interactions (Kaushik *et al*. 2016; Saha *et al*. 2018). Saha *et al*. (2018) found that fruit weight in mango was influenced by both genetic and environmental factors, with genotype accounting for 63% of the variation and environment accounting for 37%. This suggests that careful selection of genotypes based on these parameters could lead to improved yields and profitability, but also that management practices such as irrigation, fertilization, and pest control could have a significant impact on the expression of these traits. The significant variation in stone weight, fruit weight, pulp percentage, and TSS among wild mango genotypes. (Rana *et al*. 2016; Atanda *et al*. 2020).

The highest level of significance was observed in fruit diameter, pulp percentage, petiole length indicating that these parameters vary significantly among the 45 wild mango genotypes. Jaiswal *et al*. (2019) found significant variation in fruit weight, pulp weight, and TSS among different mango cultivars. Kumar *et al*. (2015) reported significant variation in fruit length, fruit width, and fruit weight among different mango cultivars. It was identified fruit diameter, pulp percentage, and petiole length as the parameters with the highest levels of significance among the studied mango cultivars. This suggests that these parameters may be particularly important in identifying and selecting mango cultivars with desirable traits for commercial production.

Breeding and selection programs can use to develop new mango cultivars with improved fruit quality and yield. The mango cultivars with larger fruit diameter can be selected for export markets where size is an important factor. The cultivars with higher pulp percentage can be selected for pulp production. The evaluation of germplasm also aids in the selection of parents for breeding programmes to increase production (Singh *et al.,* 2021). Kheshin *et al*. (2016); Vieccelli *et al*. (2016). Significant differences were observed in seed length, width, and weight among the mango cultivars.

The different plant varieties of coastal districts in Karnataka that have investigated the qualitative characteristics of mangoes. Sivakumar *et al*. (2008) on mango cultivars in India found a wide range of fruit sizes, ranging from small to large, with variations in fruit shape, color, and pulp characteristics. The variations in other qualitative traits such as acidity, sweetness, and flavor. In addition to fruit characteristics, several studies have investigated the genetic diversity and phylogenetic relationships among different mango varieties. The different mango varieties in coastal districts of Karnataka can help in identifying and preserving genetic resources. This indicates that these traits are more stable and less influenced by experimental conditions and genetic differences between the varieties. These results suggest that the variation in different traits of mangoes is not uniform. The significant variation in traits such as leaf blade length, leaf blade width, and peel percentage. (Alcazar-magna *et al*. 2018; Anjum *et al*. 2018)

Genetic factA high GCV indicates a greater amount of genetic variation in the population, while a low GCV indicates little genetic variation. It was measured several traits in a population and found that leaf blade length, petiole length, fruit weight, pulp percentage, peel percentage, fruit thickness, stone thickness, stone weight possess high GCV values. This suggests that these traits have a significant genetic component and are likely to respond well to selective breeding efforts aimed at improving them. Leaf blade width, fruit length, fruit diameter, fruit width, TSS, and stone length had medium GCV values, indicating a moderate level of genetic variation for these traits. While not as genetically variable as the high GCV traits, these medium GCV traits still have potential for improvement through breeding as well as environmental factors such as wind and light exposure.(Pandey *et al.* 2014; Sathish *et al*. 2015; Singh *et al*. 2016).

The analysis of variance (ANOVA) for each trait revealed that leaf blade length, leaf blade width, and peel percentage had significant variation in both replicates and treatments. This

indicates that these traits are strongly influenced by both the experimental conditions and inherent genetic differences between the mango varieties. The significant variation in these traits suggests that they can be selectively bred for specific desirable characteristics, such as leaf size or peel thickness. The remaining traits, including petiole length, fruit length, fruit diameter, fruit weight, fruit width, pulp, TSS, fruit thickness, stone length, stone thickness, and stone weight did not show any significant variation in either replicates or treatments.

The collection and evaluation of mango germplasm from various natural populations. Agro-morphological traits such as plant height, leaf characteristics, flowering and fruiting patterns, and fruit morphology were likely assessed to determine the variation within the populations. Quality of the fruits, such as taste, aroma, texture, and nutritional traits content. Local varieties or landraces harbor specific traits that can be exploited in improvement programs to cope with biotic and abiotic stresses and to meet consumers preferences.

Gaurav and Tomar (2020) analyzed the physico-chemical properties of mango varieties and found that the pulp of mangoes had a high percentage of moisture, TSS, and total sugar. They also observed variations in the acidity, ascorbic acid, and mineral content of different mango varieties. Zainol *et al*. (2013) investigated the antioxidant activity and phenolic content of mango fruit peel and flesh. The peel had higher antioxidant activity and phenolic content than the flesh, and both showed variations in these characteristics among different mango varieties. Siddique *et al*. (2018) examined the sensory attributes of mango cultivars and found significant differences in taste, aroma, texture, and overall acceptability among them. Zainol *et al*. (2013), which reported variations in the antioxidant activity and phenolic content of mango fruit peel among different varieties. Such variations in peel characteristics can influence the nutritional and medicinal properties of mangoes, making them an important target for breeding and selection.

1.0 INTRODUCTION

Mango (*Mangifera indica* L.) stands as one of the most economically and culturally significant tropical fruits globally, boasting a rich array of cultivars with diverse morphological, physiological and biochemical traits (Roslin and Vinayagmurthy, 2014). The elucidation of genetic diversity and relationships among mango varieties is crucial for effective breeding strategies, germplasm conservation, and sustainable management practices (Jena *et al*. 2021). While traditional methods have provided details, the application of molecular markers,

particularly Simple Sequence Repeat (SSR) or microsatellite markers, offers a high-resolution approach to dissecting genetic variation within mango populations (Duval *et al*. 2005; Hadhary *et al*. 2015; Saleem *et al*. 2018; Sarika *et al*. 2019).

SSR markers are characterized by short tandem repeats of 1-6 base pairs dispersed throughout the genome. Their co-dominant nature, multiallelic variation, high reproducibility, and abundance in the genome make them ideal for population genetic studies (Powell *et al*. 1996; Vieira *et al*. 2016). SSR markers facilitate the identification of unique alleles and the generation of informative genetic profiles within populations, thus overcoming the limitations of morphological descriptors and biochemical markers, which often fail to capture subtle genetic differences and relationships among closely related mango cultivars. (Gupta *et al*. 1999).

The application of SSR markers has significantly enhanced our understanding of genetic diversity and relationships among cultivars. Duval *et al*. (2005) developed SSR markers specifically for mango, which have been used to analyze genetic variability and structure within and among mango populations. Viruel and Hormaza (2004) characterized and applied SSR markers to assess genetic diversity in a wide range of mango cultivars.

The utilization of SSR markers has gained momentum in mango research, spurred by advancements in molecular techniques and the availability of genomic resources. Previous studies have demonstrated the utility of SSR markers in characterizing genetic diversity, population structure, and phylogenetic relationships among diverse mango germplasm collections worldwide (Viruel *et al*. 2004; Duval *et al*. 2005). These investigations have provided valuable insights into the evolutionary history, geographic origins, and genetic differentiation of mango cultivars, shedding light on their domestication processes and genetic relationships with wild relatives. The application of molecular primers in genetic studies of wild mangoes offers several significant advantages:

High resolution and specificity: SSR markers provide high-resolution genetic profiles, enabling the detection of subtle genetic differences and relationships among wild mangoes. This high specificity is crucial for identifying unique genetic variants that may be valuable for breeding and conservation (Duval *et al*. 2005).

Assessment of genetic structure: These primers facilitate detailed analyzes of population structure, allowing researchers to delineate distinct genetic clusters within wild mango

populations. Such insights are essential for understanding the evolutionary history and geographic distribution of genetic diversity (Viruel and Hormaza, 2004).

Conservation and breeding: By identifying regions of the genome associated with important traits, molecular primers aid in the conservation of genetic diversity and the selection of desirable traits for breeding programs. This ensures that valuable genetic traits are preserved and utilized to improve cultivated mango varieties (Ravishankar *et al*. 2000).

Comparative studies: The use of these primers allows for comparative studies between wild and cultivated mangoes, shedding light on domestication processes and the genetic contributions of wild relatives to cultivated varieties. This knowledge is vital for enhancing the genetic base of cultivated mangoes and improving their resilience to biotic and abiotic stresses (Pandit *et al*. 2007).

5. DISCUSSION

The genetic diversity identified would help to ensure the long-term sustainability of the mango industry in Australia. The importance of maintaining a gene bank for mangoes and gene banks are essential for the conservation of genetic diversity and the preservation of genetic resources. (Dillon *et al*. 2013)

The genetic relationships among mango accessions were examined using SSR markers a. A total of 180 alleles and 22.50 alleles per locus were examined across all mango varieties using primers. The observed heterozygosity (H_O) ranged from 0.15 to 0.63 with an average of 0.41 while the expected heterozygosity (H_E) varied from 0.57 to 0.95 with a mean of 0.83. The similar works were carried out by Ng *et al.* (2017). The low level of genetic variability by inbreeding within the populations and different clusters indicates the presence of genetic differentiation among the populations (Hadhary *et al*. 2015).

Cluster analysis of the mango cultivars based on their AFLP profiles showed two major groups, indicating some degree of genetic differentiation among the cultivars. (Rahman *et al.* 2022).They found that the populations were genetically diverse and suggested that this could be due to the influence of environmental factors such as soil type and climatic conditions. Ravishankar *et al.* (2004), the genetic relatedness among polyembryonic and monoembryonic mango cultivars traditionally grown on the West coast of Southern India using RAPD markers was studied.

The data associated with the assessment of the genetic diversity of 42 mango varieties collected in the present study Cluster 1: Kelakila, Thacode 1, Laadi, and Pilimogaru. This cluster indicates a high level of genetic diversity within the group, suggesting that these varieties share a common genetic background but also exhibit considerable genetic variation. Cluster 2:Hebri, Adishakthi mudradi, Mudradi, Bailumane-1, Sitanadi, Sitanadi,Yennehole, Jodurasthe, Miyar kuntibail, Sampige, Thacode 2, Alangar, Birav, Ontikatte, Dhande, Kodiyadka, Siddakatte,

Nelyakumar.Cluster 3: Muniyal, Koragatte. These two varieties are closely related to each other but distinct from the other clusters. Cluster 4: Bailumane 2, Jodukatte, Anekere, Pulkeri, Borugudde, Bannadka, Beluvayi, Meginamane, Papermill, Kodiyadka, Karimale, Ananthady, korey, Kuthkoli, Padil. Cluster 5: Masthikatte, Karkala bypass and it represents another significant group of genetically similar varieties, indicating a shared lineage among these mango varieties. The similar work carried Joshi *et al*. (2016) and concluded that relying solely on morphological traits may not be sufficient to differentiate between different varieties of mangoes. Ashfaq *et al*. (2017) analyzed the data, including cluster analysis, genetic diversity analysis, and principal coordinate analysis (PCoA). Cluster analysis based on the genetic similarity coefficients revealed four distinct clusters, with some samples showing a high degree of genetic similarity within the clusters. The genetic diversity analysis showed that the observed heterozygosity ranged from 0.04 to 0.67, and the expected heterozygosity ranged from 0.16 to 0.71, indicating a wide range of genetic diversity among the wild mango varieties from Pakistan. The PCoA results showed that the first two principal coordinates accounted for 51.6% of the total variation, indicating a high level of genetic diversity among the samples. Sarika *et al*. (2019) assessed the clustering analysis revealed a clear separation of the mango varieties into three major groups based on their geographical origin.

The dendrogram analysis showed coefficients of similarity ranging from 75 to 100%, indicating the frequent use of only a few parent cultivars and the presence of inbreeding. The identification of inbreeding among these cultivars highlights the need for improved breeding strategies to enhance genetic diversity and maintain the long-term sustainability of mango cultivation. (Nazish *et al*. 2017).

The data. Coord. 1 explains 8.92% of the variance and Coord. 2 explains 7.19% of the variance. Together, these two coordinates explain 16.11% of the total variance in the dataset. A large number of observations are clustered around the center, indicating they have similar characteristics. Examples include Sitanadi, Bailumane-1, and Iravaje. Hasan *et al*. (2022) assessed the genetic diversity of 38 mango cultivars from Bangladesh using SSR markers. They collected young leaves from the cultivars and extracted genomic DNA, which was used to amplify 20 SSR loci. The results showed that all 20 SSR loci were polymorphic, with a total of 116 alleles detected across the cultivars. The mean number of alleles per locus was 5.8, and the mean observed and expected heterozygosity were 0.38 and 0.61, respectively. They used

principal coordinate analysis (PCoA) and unweighted pair-group method with arithmetic means (UPGMA) clustering to evaluate the genetic relationships among the mango cultivars. It was showed that the cultivars could be grouped into two main clusters, with a moderate level of genetic diversity observed within and between the clusters. The moderate level of genetic diversity as the prevalence of vegetative propagation and the widespread cultivation of a few popular cultivars.

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(Dr Rama Bhat P.)
Research Guide

(Ruchika)
Research Scholar

LIST OF RESEARCH PUBLICATIONS

1. Ruchika, Bhat PR. and Ravishankar K.V. (2023).Qualitative and Quantitative assesment of the characters of wild varieties of Mango (*Mangifera indica* L.) in coastal Districts of Karnataka. *Journal of Horticultural Sciences 18*(2): 271-275.

2. Ruchika, Rama Bhat P. and Ravishankar K.V. (2023). Assesment of genetic variation of wild varieties of Mango (*Mangifera indica* L.) of the Western Ghats by molecular markers. *Solovyov Studies* (ISPU) *71*(10): 31-44.

LIST OF PRESENTATIONS

1. The oral presentation on Assessment of Genetic diversity of wild varieties of Mango (*Mangifera indica* L.) of Western Ghats using molecular markers in the National Conference on "Advances in Biotechnology and Nanotechnology" at Karnataka University, Dharwad on March 14th and 15th 2023.

2. The oral presentation on Qualitative and Quantitative assesment of the characters of wild varieties of Mango (*Mangifera indica* L.) in coastal Districts of Karnataka in the

“International Conference on Current Technologies and opportunities in Bio-Science” at College of Agriculture, Karekere Hassan held during March 27th to 29th 2023.